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Unsolved problems in congenital diaphragmatic hernia

Edited by
Dick Tibboel, Anne Greenough, Neil Patel and Thomas Schaible

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Unsolved problems in congenital diaphragmatic hernia

Topic editors

Dick Tibboel — Erasmus Medical Center, Netherlands

Anne Greenough — King's College London, United Kingdom

Neil Patel — Royal Hospital for Children, United Kingdom

Thomas Schaible — University Medical Centre Mannheim, University of Heidelberg, Germany

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Table of contents

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Eugene Dempsey,
University College Cork, Ireland

*CORRESPONDENCE
Dick Tibboel
✉ d.tibboel@erasmusmc.nl

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Editorial: Unsolved problems in congenital diaphragmatic hernia

Dick Tibboel[1,2]*, Anne Greenough[3,4,5], Neil Patel[6], Pietro Bagolan[7,8] and Thomas Schaible[9]

[1]Intensive Care, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands, [2]Pediatric Surgery, Erasmus MC, Sophia Children's Hospital, University Medical Center Rotterdam, Rotterdam, Netherlands, [3]Department of Women and Children's Health, School of Life Course Sciences, King's College, London, United Kingdom, [4]Department of Women and Children's Health, School of Life Course Sciences, King's College London, London, United Kingdom, [5]Neonatal Intensive Care Unit, King's College Hospital, London, United Kingdom, [6]Department of Neonatology, Royal Hospital for Children, Glasgow, United Kingdom, [7]Neonatal Surgery Unit, Area of Fetal, Neonatal and Cardiological Sciences, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy, [8]Department of Systems Medicine, University of Tor Vergata, Rome, Italy, [9]Department of Neonatology, Universitätsklinikum Mannheim, Mannheim, Germany

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Editorial on the Research Topic
Unsolved problems in congenital diaphragmatic hernia

Introduction

Congenital diaphragmatic hernia (CDH) remains a major congenital anomaly with high mortality and long-term morbidity with many unsolved aspects ranging from the cause to the optimal way of follow-up. Major progress has been made over the last few decades to identify the underlying cause, prenatal therapeutic approaches, neonatal care with regard to optimal ventilatory strategies and treatment of pulmonary hypertension, and evaluation of both surgical and non-surgical morbidities.

Even in the most reputed centers that depend on case mix with only referrals, the availability of extracorporeal membrane oxygenation (ECMO), and a pathophysiological understanding of the role of heart dysfunction, the mortality rate continues to be approximately 20%–30%. Experienced medical teams will acknowledge that in CDH, there are "good and bad years" (like wine) in evaluating mortality in individual clinics.

International collaborations are very important as reflected by the CDH EURO Consortium (guidelines; clinical trials); the International CDH Study Group (epidemiology; risk assessment); DHREAMS (genetics). International therapy guidelines have been published both in Europe and in Canada and Japan. Unfortunately, these provide a low level of evidence even today (1–3).

This important series, Unsolved Problems in CDH, has brought together contributions from established and emerging leaders in the field, from basic science, through fetal therapies, and neonatal pathophysiology to optimized long-term management. Although much remains uncertain and challenges persist, this series provides a "state of the art" of current understanding and identifies key priorities for future research and advancing clinical care in CDH.

Genetics

Since CDH is a relatively rare disorder with only a few recurrent changes, large cohorts of patients are needed to identify genetic associations. Retrospective whole-genome sequencing of historical patient cohorts will yield valuable data from which the patients of today and tomorrow will profit. Trio whole-genome sequencing has an excellent potential for future re-analysis and data sharing, increasing the opportunity to provide a genetic diagnosis and predict clinical prognosis (Brosens et al.). The success of this effort stresses the importance of collaboration such as within the DHREAMS consortium (http://www.cdhgenetics.com).

Increased insights into the pathogenesis and combination of different congenital anomalies (Gaillard et al.) will be of value to identify specific pathways involved in lung and diaphragm development, together with a profound knowledge of the factors determining (ab)normal lung and diaphragm development (Edel et al.).

Instead of trying to identify "THE CDH gene", nowadays, disturbances in specific pathways are investigated in more detail (4).

Prenatal therapy

Advances in prenatal genetic evaluation to guide optimal prenatal counseling have been accompanied by many years of hard work to investigate the impact of fetal intervention. On the other hand, factors associated with the decision for termination of pregnancy (TOP) are additional fetal genetic or anatomical abnormalities and expected severity of pulmonary hypoplasia in left-sided CDH (5).

An international group guided by the Leuven team recently published data on two sets of temporary prenatal occlusion of the human fetal trachea (TOTAL trial) (6, 7). They observed no significant differences in patients with moderate CDH but a significant survival advantage in a group of patients with severe CDH fetuses and who received prenatal tracheal occlusion. This finding will have important consequences for the implementation of prenatal tracheal plugging around the world with debates centered around centralization of this procedure. A high level of and standardization of prenatal diagnosis with either ultrasound or MRI is pivotal for future patient inclusion and for inclusion as a subject of international training courses (8).

The first breath

Based on animal experiments and evaluation, the first breath and changes in lung inflation and the circulation at transition have resulted in two large randomized trials—Congenital Hernia Intact Cord (CHIC, NCT04429750) (9) and Physiological-based cord clamping for infants with a Congenital Diaphragmatic Hernia (PinC, NCT04373902) (DeKoninck et al.) (10)—to evaluate the effect of delayed, physiology-based cord clamping. Short-term outcomes such as Apgar scores (CHIC) and pulmonary hypertension (PinC) have been selected as primary outcomes. The results are expected in 2–3 years' time.

International guidelines such as those published over the last decade both by the CDH EURO Consortium and by the Canadian and Japanese study groups advise intubation for every newborn with prenatally diagnosed CDH. Taking into account the potential negative effects of this approach, people have now started questioning this approach, resulting in pilot data on spontaneous breathing in select CDH cases (11). An international collaboration will now evaluate this protocol in more patients on the basis of a proposed algorithm for spontaneous breathing. A value of the observed expected (OE)/ lung to head ratio (LHR) of above 50, although arbitrary, is chosen as the cutoff to be included in this study.

Respiratory support

The optimal method of ventilatory support remains a subject of debate and the same holds true for the application of ECMO in select cases. Although the VICI trial (12) concluded that conventional ventilation (CV) was superior to high frequency oscillation (HFO), this only implies the initial ventilatory mode and also begs the question, "Which CV mode? It is vital that lung-protective ventilation strategies are employed during both initial stabilization and postsurgical repair to avoid ventilator-induced lung damage and oxygen toxicity to prevent further impairment to an already diminished gas-exchanging environment. In this context, clinicians continue to investigate predictive parameters as markers for mortality and morbidity, such as the NeoAPACHE II score and the chest radiographic thoracic area (Amodeo et al.; Weis et al.).

It would be important to evaluate closed-loop automated oxygen control, which is the reinvention of liquid ventilation and heliox therapy in properly designed clinical trials with international collaboration (Williams and Greenough).

Not only differences in mortality but increasingly the incidence and magnitude of chronic respiratory morbidity should be considered primary endpoints in these studies. Objective criteria for lung function both in the acute phase and during child and adulthood are fundamental to any decision to implement future treatment algorithms. In this way, we can establish a state-of-the-art evaluation of the lungs at different time points in life to understand the overall effects on the hypoplastic lungs and the secondary damage by ventilator-induced lung injury (VILI).

Extracorporeal membrane oxygenation

In select cases, many clinics start ECMO as a rescue therapy, although the overall effects on survival remain debatable. Despite its wide use and decades of experience, the survival rate of CDH patients treated with ECMO, as reported by the extracorporeal life support organization (ELSO), remains unchanged at 50% (13). This is probably due to negative case selection. Individual

centers report higher survival rates of up to 70%, such as the Mannheim group (Germany), and can be classified as best practices for this specific treatment modality (14). ELSO data analysis also shows that ECMO improves survival rates in those CDH patients who are most severely affected, but the potential complications of ECMO delivery outweigh the benefits in less severely affected patients. The large variability in ECMO survival rate is determined by preferences such as the mode of ECMO (VV vs. VA), timing of ECMO (early vs. late), patient selection (inclusion criteria variability), surgery (on or after ECMO), supportive cardiac therapy (iNO vs. milrinone), and outcome parameters (alive at decannulation vs. alive at discharge). At present, there is no single test or prognostication that predicts the reversibility of primary pulmonary hypertension of the newborn (PPHN), and the criteria for referral for ECMO are under the process of continued refinement. Therefore, the real contribution of ECMO needs to be investigated in a properly designed RCT using Bayesian statistical approaches.

The role of the heart in CDH

Postnatal clinical care in CDH has traditionally viewed the lungs as the primary "defective" organs; however, there is growing recognition of the important pathophysiological role played by the heart in CDH.

The coexistence of major cardiac anomalies is an important factor determining survival in CDH and a key consideration in decision making with families of patients in the pre- and postnatal periods.

Pathology specimen and cardiac ultrasound–based studies have additionally identified fetal left ventricular hypoplasia in CDH fetuses. In combination with the established abnormalities of the pulmonary vasculature, this developmental abnormality of the circulation likely contributes to the failed transition at birth, of which variable right and left ventricular dysfunction are key components and determinants of disease severity and outcome (Patel et al.). Although clinical studies have identified the nature of ventricular dysfunction in CDH, the underlying cellular, metabolic, and genetic contributions remain unknown and an important area for investigation.

In the clinical setting, further studies are also required to understand the benefits of routine echocardiographic evaluation of cardiac function, utilizing predetermined and internationally accepted parameters, and the role of this approach in guiding evidence-based therapy of individual patient pathophysiology.

This targeted therapeutic approach, combined with a better understanding of the model-based pharmacokinetic dosing, may lead to a more effective use of well-known and frequently used hemodynamic agents such as iNO, IV sildenafil, milrinone, and PGE1 (Hari Gopal et al.). Unfortunately, the so-called CODINOS trial of iNO and IV sildenafil (15) (Cochius-den Otter et al.) was stopped recently because of a very low inclusion rate and the practical challenges of performing multicenter, investigator-initiated drug studies. The burden of regulatory rules and financial implications underlines the major challenges of performing any clinical trials in CDH.

Surgery

Closure of the diaphragmatic defect as a semielective surgical procedure is nowadays accepted worldwide. It is an important step in the treatment algorithm incorporating careful planning to prevent intraoperative complications related to hemodynamic instability and recurrent pulmonary hypertension in particular. The defects of most patients with defect sizes of A and B (Boston classification) can be closed primarily without a patch either by an open or a minimally invasive technique, resulting in a change from an abdominal to a thoracic approach. In the latter, CO_2 inflation is warranted, which might alter the metabolic balance, resulting in pulmonary hypertensive crisis. Less than 10% of newborns will die unoperated almost exclusively of a type D defect (agenesis of the diaphragm) because of a bad prognosis making surgery futile. Importantly, some patients do not classify for surgery already during fetal evaluation. In this group, the main factors are additional fetal genetic or anatomical abnormalities and expected severity of pulmonary hypoplasia in left-sided CDH based on fetal O/E LHR obtained by ultrasound and/or lung volumes by MRI.

As part of a routine longitudinal follow-up, recurrence of the diaphragmatic defect is detected even if cone-shaped patches are used (Zahn et al.; Macchini et al.). The major and most frequently reported downside of minimally invasive surgery in CDH repair is the higher risk of recurrence, reported three- to four-fold higher following an minimal invasive surgery (MIS) approach.

Apart from the original diaphragmatic defect, a long-term follow-up shows the occurrence of hiatal hernia, resulting in gastro-intestinal (GI)- and pulmonary-related morbidity. A longitudinal follow-up with regular radiologic imaging until adolescence is essential to reliably detect recurrence to prevent acute incarceration and chronic gastrointestinal morbidity such as the occurrence of the small bowel obstruction, which may lead to clinical symptoms at any age with serious consequences (Zahn et al.) and impact prognosis.

Optimal timing for repair during ECMO (early vs. late; never on ECMO vs. always; etc.) is still a matter of debate, given the potential bleeding complications during the procedure. No properly designed RCTs are available yet. The same holds true for the choice of the biological or synthetic patch material.

Palliative care for patients, both fetuses and neonates (and their families), who are identified as untreatable because of CDH or associated severe anomalies, is also a future option to consider as part of quality of care.

Follow-up

The lower mortality rates of CDH patients go hand in hand with the need for paying more attention to the long-term morbidity of different organ systems. Apart from the "classical" organs such as the lungs and the GI tract, a structured long-term follow-up is being offered nowadays by an increasing number of centers. High-volume centers in Rotterdam, the Netherlands,

Mannheim, Germany, Rome, and Italy, among others, have published their schedules and results over the years on a variety of aspects (3, 16–18). They use their prospective and highly standardized databases related to the evolution from newborns to adolescence (de Munck et al.; Valfré et al.).

Neurodevelopmental outcome is an important determinant for the future. This has resulted in the concept of "growing into deficit" (19), in which specific higher-executive functions (short-term memory, task performing) have been identified as abnormal and last into adulthood. Prospective longitudinal evaluation combining neuropsychological tests with neuro-imaging are needed for developing a full understanding of these abnormalities and implementation of targeted therapies.

It is very important for comparative analysis that reference values are used on the basis of the respective populations in different countries. Particularly relevant for follow-up are guidelines that are developed in close collaboration between the CDH EURO consortium and ERNICA (European Reference Network for rare Inherited and Congenital Anomalies). ERNICA is a network of expert multidisciplinary healthcare professionals from specialized healthcare providers across Europe. Their aim is to pull together disease-specific expertise, knowledge, and resources otherwise unachievable in a single country.

The involvement of parents in many aspects of care is fundamental to identifying the "real questions" that these families have to contend with on a daily basis. The recent CDH-UK patient journey published as part of this series is a good example of collaboration between healthcare providers and parents to enhance mutual understanding and is of great significance (Power).

Synthesis

Molecular genetic analysis, combined with an understanding of the developmental pathways of the lungs and diaphragm, will result in an enhanced knowledge of the causes of CDH in individual cases with potential important consequences for fetal interventions. International guidelines for the performance of fetal US/MRI in the context of international collaboration are also pivotal.

Further research is required to identify the optimum method of respiratory support for CDH patients, which is the least damaging to their vulnerable lungs as judged by a reduction in chronic respiratory morbidity. Essential to the success of such research is internationally collaborative randomized controlled trials.

There needs to be an improved understanding of the role of the heart in CDH, combining cellular and clinical studies, incorporating cardiac parameters into fetal predictive scores, and exploring the benefits of pathophysiology-based cardiorespiratory management strategies, from the transitional period onward.

A comparative analysis of internationally available (open access) follow-up databases opens the opportunity for intervention studies aiming to reduce long-term morbidity in different organ systems.

The ultimate aim is to increase survival rates at the lowest level of morbidity. This is a real challenge for all those who are confronted with this very special group of patients who are a significant burden for their parents upon a diagnosis of CDH either during the prenatal stage or as newborns. These patients deserve our lifelong attention and care both from a somatic and from a psychosocial point of view.

Author contributions

All authors worked on the content of this editorial and critically reviewed it and fully support its publication. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Snoek KG, Reiss IK, Greenough A, Capolupo I, Urlesberger B, Wessel L, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH EURO consortium consensus—2015 update. *Neonatology*. (2016) 110(1):66–74. doi: 10.1159/000444210

2. LaRusso K, Baird R, Keijzer R, Skarsgard E, Puligandla P. Standardizing congenital diaphragmatic hernia care in Canada: implementing national clinical practice guidelines. *J Pediatr Surg*. (2020) 55(5):835–43. doi: 10.1016/j.jpedsurg.2020.01.021

3. Lally PA, Skarsgard ED. Congenital diaphragmatic hernia: the role of multi-institutional collaboration and patient registries in supporting best practice. *Semin Pediatr Surg*. (2017) 26(3):129–35. doi: 10.1053/j.sempedsurg.2017.04.004

4. Donahoe PK, Longoni M, High FA. Polygenic causes of congenital diaphragmatic hernia produce common lung pathologies. *Am J Pathol*. (2016) 186(10):2532–43. doi: 10.1016/j.ajpath.2016.07.006

5. Horn-Oudshoorn EJJ, Peters NCJ, Franx A, Eggink AJ, Cochius-den Otter SCM, Reiss IKM, et al. Termination of pregnancy after a prenatal diagnosis of congenital diaphragmatic hernia: factors influencing the parental decision process. *Prenat Diagn*. (2023) 43(1):95–101. doi: 10.1002/pd.6274

6. Deprest JA, Nicolaides KH, Benachi A, Gratacos E, Ryan G, Persico N, et al. Randomized trial of fetal surgery for severe left diaphragmatic hernia. *N Engl J Med*. (2021) 385(2):107–18. doi: 10.1056/NEJMoa2027030

7. Deprest JA, Benachi A, Gratacos E, Nicolaides KH, Berg C, Persico N, et al. Randomized trial of fetal surgery for moderate left diaphragmatic hernia. *N Engl J Med*. (2021) 385(2):119–29. doi: 10.1056/NEJMoa2026983

8. Russo FM, Cordier AG, De Catte L, Saada J, Benachi A, Deprest J, Workstream Prenatal Management, ERNICA European Reference Network. Proposal for standardized prenatal ultrasound assessment of the fetus with congenital diaphragmatic hernia by the European reference network on rare inherited and congenital anomalies (ERNICA). *Prenat Diagn*. (2018) 38(9):629–37. doi: 10.1002/pd.5297

9. Duc K, Mur S, Rakza T, Boukhris MR, Rousset C, Vaast P, et al. Efficacy of intact cord resuscitation compared to immediate cord clamping on cardiorespiratory adaptation at birth in infants with isolated congenital diaphragmatic hernia (CHIC). *Children (Basel)*. (2021) 8(5):339. doi: 10.3390/children8050339

10. Kashyap AJ, Hodges RJ, Thio M, Rodgers KA, Amberg BJ, McGillick EV, et al. Physiologically based cord clamping improves cardiopulmonary haemodynamics in lambs with a diaphragmatic hernia. *Arch Dis Child Fetal Neonatal Ed*. (2020) 105 (1):18–25. doi: 10.1136/archdischild-2019-316906

11. Cochius-den Otter SCM, Horn-Oudshoorn EJJ, Allegaert K, DeKoninck PLJ, Peters NCJ, Cohen-Overbeek TE, et al. Routine intubation in newborns with congenital diaphragmatic hernia. *Pediatrics*. (2020) 146(4):e20201258. doi: 10.1542/peds.2020-1258

12. Snoek KG, Capolupo I, van Rosmalen J, Hout Lde J, Vijfhuize S, Greenough A, et al. Conventional mechanical ventilation versus high-frequency oscillatory ventilation for congenital diaphragmatic hernia: a randomized clinical trial (the VICI-trial). *Ann Surg*. (2016) 263(5):867–74. doi: 10.1097/SLA.0000000000001533

13. Kays DW. ECMO in CDH: is there a role? *Semin Pediatr Surg*. (2017) 26 (3):166–70. doi: 10.1053/j.sempedsurg.2017.04.006

14. Rafat N, Schaible T. Extracorporeal membrane oxygenation in congenital diaphragmatic hernia. *Front Pediatr*. (2019) 7:336. doi: 10.3389/fped.2019.00336

15. Cochius-den Otter S, Schaible T, Greenough A, van Heijst A, Patel N, Allegaert K, et al. The CoDiNOS trial protocol: an international randomised controlled trial of intravenous sildenafil versus inhaled nitric oxide for the treatment of pulmonary hypertension in neonates with congenital diaphragmatic hernia. *BMJ Open*. (2019) 9(11):e032122. doi: 10.1136/bmjopen-2019-032122

16. Morini F, Valfrè L, Bagolan P. Long-term morbidity of congenital diaphragmatic hernia: a plea for standardization. *Semin Pediatr Surg*. (2017) 26(5):301–10. doi: 10.1053/j.sempedsurg.2017.09.002

17. IJsselstijn H, Breatnach C, Hoskote A, Greenough A, Patel N, Capolupo I, et al. Defining outcomes following congenital diaphragmatic hernia using standardised clinical assessment and management plan (SCAMP) methodology within the CDH EURO consortium. *Pediatr Res*. (2018) 84(2):181–9. doi: 10.1038/s41390-018-0063-3

18. Van der Veeken L, Vergote S, Kunpalin Y, Kristensen K, Deprest J, Bruschettini M. Neurodevelopmental outcomes in children with isolated congenital diaphragmatic hernia: a systematic review and meta-analysis. *Prenat Diagn*. (2022) 42(3):318–29. doi: 10.1002/pd.5916

19. Schiller R, IJsselstijn H, Hoskote A, White T, Verhulst F, van Heijst A, et al. Memory deficits following neonatal critical illness: a common neurodevelopmental pathway. *Lancet Child Adolesc Health*. (2018) 2(4):281–9. doi: 10.1016/S2352-4642(17)30180-3

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NeoAPACHE II. Relationship Between Radiographic Pulmonary Area and Pulmonary Hypertension, Mortality, and Hernia Recurrence in Newborns With CDH

Ilaria Amodeo[1†], *Nicola Pesenti*[1,2†], *Genny Raffaeli*[1,3], *Francesco Macchini*[4], *Valentina Condò*[1], *Irene Borzani*[5], *Nicola Persico*[3,6], *Isabella Fabietti*[6], *Giulia Bischetti*[1], *Anna Maria Colli*[7], *Stefano Ghirardello*[1,8], *Silvana Gangi*[1], *Mariarosa Colnaghi*[1], *Fabio Mosca*[1,3] *and Giacomo Cavallaro*[1*]

[1] *Neonatal Intensive Care Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy,* [2] *Division of Biostatistics, Epidemiology and Public Health, Department of Statistics and Quantitative Methods, University of Milano-Bicocca, Milan, Italy,* [3] *Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Milan, Italy,* [4] *Department of Pediatric Surgery, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy,* [5] *Pediatric Radiology Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy,* [6] *Department of Obstetrics and Gynecology, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy,* [7] *Cardiology Department, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy,* [8] *Neonatal Intensive Care Unit, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy*

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Thomas Schaible, University of Heidelberg, Germany

Reviewed by:
Carmen Mesas Burgos, Karolinska Institutet (KI), Sweden
Arno Van Heijst, Self-Employed, Millingen aan de Rijn, Netherlands

***Correspondence:**
Giacomo Cavallaro
giacomo.cavallaro@policlinico.mi.it

[†]These authors share first authorship

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Congenital diaphragmatic hernia is a rare disease with high mortality and morbidity due to pulmonary hypoplasia and pulmonary hypertension. The aim of the study is to investigate the relationship between radiographic lung area and systolic pulmonary artery pressure (sPAP) on the first day of life, mortality, and hernia recurrence during the first year of life in infants with a congenital diaphragmatic hernia (CDH). A retrospective data collection was performed on 77 CDH newborns. Echocardiographic sPAP value, deaths, and recurrence cases were recorded. Lung area was calculated by tracing the lung's perimeter, excluding mediastinal structures, and herniated organs, on the preoperative chest X-ray performed within 24 h after birth. Logistic and linear regression analyses were performed. Deceased infants showed lower areas and higher sPAP values. One square centimeter of rising in the total, ipsilateral, and contralateral area was associated with a 22, 43, and 24% reduction in mortality risk. sPAP values showed a decreasing trend after birth, with a maximum of 1.84 mmHg reduction per unitary increment in the ipsilateral area at birth. Recurrence patients showed lower areas, with recurrence risk decreasing by 14 and 29% per unit increment of the total and ipsilateral area. In CDH patients, low lung area at birth reflects impaired lung development and defect size, being associated with increased sPAP values, mortality, and recurrence risk.

Clinical Trial Registration: The manuscript is an exploratory secondary analysis of the trial registered at ClinicalTrials.gov with identifier NCT04396028.

Keywords: congenital diaphragmatic hernia, radiographic lung area, lung hypoplasia, pulmonary hypertension, mortality, recurrence of the hernia, FETO

INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a severe congenital malformation with a wide outcome variability (1). Pulmonary hypoplasia and persistent pulmonary hypertension (PH) represent the two main determinants of outcomes in patients, with still high mortality and morbidity (2–12). The radiographic assessment of the lung area has been proposed as an alternative method to evaluate pulmonary hypoplasia soon after birth (13–15). In newborns with CDH, lung area is correlated to the functional residual capacity measured through the diluted helium technique, and its increase is associated with tidal volume improvement in the first year of life (16, 17). The chest radiographic thoracic area (CRTA) was found to be lower in patients with poor prognosis and to predict survival to discharge from the Neonatal Intensive Care Unit (NICU) better than lung-to-head ratio at diagnosis (LHR) (18). However, a possible association between lung area and pulmonary hypertension has never been investigated.

Hernia recurrence represents one of the most common complications, and a large diaphragmatic defect is one of the main independent risk factors (19–25). The recurrence could occur weeks, months, or even years after the primary surgery, and patients often remain asymptomatic for a long time or until complications arise. Therefore, the overall risk of recurrence during the lifespan remains unknown (8). To our knowledge, an association between lung area and hernia recurrence has never been reported so far.

Since lung hypoplasia and vascular development are strictly related, our hypothesis was that lower lung areas at birth could determine higher mortality and higher pulmonary artery pressure (25–27). Moreover, we supposed that low lung area could indirectly reflect a large diaphragmatic defect size and be therefore associated with hernia recurrence (16, 18).

METHODS

The present study was carried out in accordance with the principles of good clinical practice and the Helsinki Declaration, as well as the national legislative and administrative provisions in force. This study was approved by the local Ethics Committee (Milan Area 2, Italy) with approval number OSMAMI-04/05/2020-0015998-U. Due to the retrospective nature of the study, the informed consent was waived by the Ethics Committee.

This study represents an exploratory secondary analysis of a previous retrospective cohort study called Assessment of the Pulmonary Area in Newborns with Congenital diaphragmatic HErnia (NeoAPACHE), performed at NICU of Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, on CDH patients over a 6-year period (January 2012–December 2018). A comprehensive description of the main study design has been previously published (NeoAPACHE I) (17).

In NeoAPACHE II, we aimed to evaluate the relationship between radiographic pulmonary area assessed on the first day of life and:

1. Pulmonary hypertension at birth, indirectly estimated by measuring the sPAP through tricuspid valve regurgitation gradient with echocardiogram;
2. Death during the first year of life; and
3. Recurrence of CDH among survivors at 1 year of life.

Moreover, the radiological features and outcomes of neonates candidate to FETO procedure were described and compared with those of the expectantly managed patients. At our Center, FETO was offered to fetuses with severe lung hypoplasia, defined as an O/E LHR <25 and <45% in left and right CDH, respectively, in the absence of major associated malformations and/or genetic anomalies known to have a significant impact on postnatal survival (28, 29).

Subjects

All CDH patients admitted to our NICU are managed according to the CDH EURO Consortium Consensus guidelines (30). As previously described, we enrolled all newborns having a preoperative chest X-ray performed within 24 h after birth at our NICU. Death within 1 h, rotated, and air leak radiographs were excluded (17). Surgery was performed as soon as the patient achieved the hemodynamic and respiratory stability through median laparotomy with either primary repair or Gore-Tex® patch insertion. According to CDHSG defect size classification, patching was performed in case of large defects (type C or D) (30–34). After discharge, all patients were included in a multidisciplinary follow-up program. In our Unit, we prefer to perform a chest X-ray at all time points during the first 2 years of life, then annually, aiming to detect asymptomatic recurrences early (8, 30, 35).

Assessment of Radiographic Pulmonary Area

Each patient's pulmonary area was assessed by freehand tracing of the diaphragm and rib cage's perimeter, excluding the mediastinal structures and herniated organs (17). If the anatomy was particularly disrupted, only the aerated portion of the lung was considered. The corresponding area was automatically calculated by the software Synapse PACS (FUJIFILM Medical Systems USA, Inc.). On each radiogram, three measurements were performed:

1. Ipsilateral pulmonary area (cm^2);
2. Contralateral pulmonary area (cm^2); and
3. Total pulmonary area (cm^2), obtained as the sum of the preceding two.

Data Collection

Data regarding prenatal history, clinical, and surgical course were collected from each patient's electronic medical records. Hernia severity was defined according to the combined evaluation

Abbreviations: AUC, area under the curve; CDH, congenital diaphragmatic hernia; CRTA, chest radiographic thoracic area; ECMO, extracorporeal membrane oxygenation; FETO, fetal endoscopic tracheal occlusion; NICU, neonatal intensive care unit; O/E LHR%, observed/expected lung-to-head ratio; PH, pulmonary hypertension; ROC, receiver operating characteristic; sPAP, systolic pulmonary artery pressure.

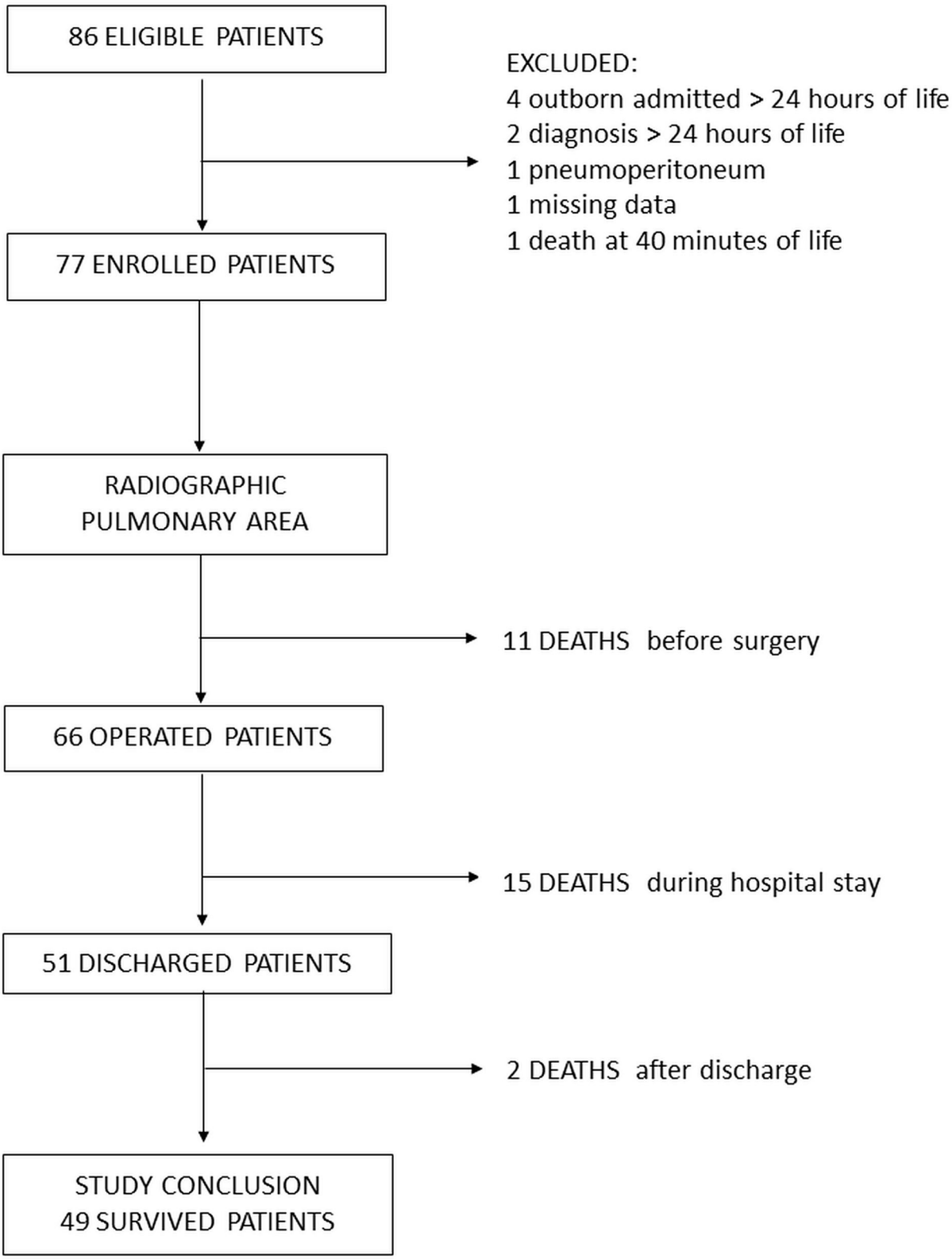

FIGURE 1 | Study flowchart.

of observed/expected lung-to-head ratio (O/E LHR%), liver herniation, and side of the diaphragmatic defect (left, right, bilateral) (2, 28, 29). Echocardiograms performed after birth (T0), pre-surgery (T1), post-surgery (T2), and 7 days after surgery (T3) were reviewed, and reported sPAP values were recorded. CDH recurrence after surgical repair and the number of deaths in the first year of life were considered. Data acquisition was anonymous.

Statistical Analysis

Continuous variables were reported as mean (standard deviation) or median (interquartile range); categorical variables were presented as number and percentage. For the comparison between groups, Student's *t*-test, Mann-Whitney *U*-test, or Fisher exact test were applied as appropriate.

The reproducibility of the method has already been assessed in the primary analysis, using the Bland Altman plot and calculating the Pearson correlation index (17).

Logistic regression models were used to evaluate the relationship between the lung area and death or hernia recurrence risk. Linear regression models were used to assess the effects of lung area on sPAP values. The models were corrected for gestational age at birth, as this variable could independently influence the lung development and survival of patients.

The ROC curve was also calculated to assess the discriminatory capacity of the radiographic measurement, thus analyzing the sensitivity and specificity of the test.

Statistical analysis was performed using IBM SPSS® Statistics V26.0. A *p*-value of 0.05 or lower was considered to be statistically significant.

Data Availability

The manuscript illustrates the results of an exploratory secondary analysis of the principal study NeoAPACHE I, registered at the ClinicalTrials.gov with identifier NCT04396028. Datasets generated during and/or analyzed during the current study are available from the corresponding author.

RESULTS

The radiographic pulmonary area was assessed on 77 patients, 49 of whom survived to discharge and were alive at the age of 1 year (36.4% mortality rate) (**Figure 1**). The majority of CDH were left-sided, with a high prevalence of severe forms and liver herniation. Fetal endoscopic tracheal occlusion (FETO) was performed in one-third of the cases, while extracorporeal membrane oxygenation (ECMO) was required in three patients. In more than half of cases, a diaphragmatic patch was needed for surgical repair, and in one patient, an abdominal patch was also used (**Table 1**).

TABLE 1 | Characteristics of the study population.

CDH (*n* = 77)	
Prenatal data	
Side of defect—*n* (%)	
- Left CDH	61 (79.2)
- Right CDH	15 (19.5)
- Bilateral CDH	1 (1.3)
O/E LHR%—mean (*SD*)	
- Initial	35.3 (12.7)
- Final	49.4 (15.7)
Liver up—*n* (%)	51 (66.2)
Grading CDH—*n* (%)	
- Severe	32 (41.6)
- Moderate	13 (16.9)
- Mild	32 (41.6)
FETO—*n* (%)	28 (36.4)
Postnatal data	
Gestational age (weeks)—mean (*SD*)	36.6 (2.2)
Birth weight (g)—mean (*SD*)	2744 (586)
Males—*n* (%)	43 (55.8)
Inborn—*n* (%)	74 (96.1)
Vaginal delivery—*n* (%)	40 (51.9)
APGAR 1 min—median (IQR)	6 (4–7)
APGAR 5 min—median (IQR)	8 (7–9)
Surgery—*n* (%)	66 (85.7)
Day of surgical repair—median (IQR)	3 (2–4)
Diaphragmatic patch (on operated)—*n* (%)	34 (51.5)
Abdominal patch (on operated)—*n* (%)	1 (1.5)
Mechanical ventilation (days)—median (IQR)	11 (7–20.5)
ECMO—*n* (%)	3 (3.9)
Length of stay (days)—median (IQR)	39 (15–68)
Deceased—*n* (%)	28 (36.4)
Radiographic pulmonary area	
Total pulmonary area (cm^2)—mean (*SD*)	12.6 (7.0)
Ipsilateral pulmonary area (cm^2)—mean (*SD*)	3.9 (3.5)
Contralateral pulmonary area (cm^2)—mean (*SD*)	8.6 (4.3)

CDH, congenital diaphragmatic hernia; ECMO, extracorporeal membrane oxygenation; FETO, fetal endoscopic tracheal occlusion; IQR, interquartile range; n: number; O/E LHR, observed/expected lung-to-head ratio; SD, standard deviation.

Radiographic Pulmonary Area, Pulmonary Hypertension, and Mortality

The study population was divided into two groups, deceased ($n = 28$) and survived ($n = 49$). Compared with survivors, deceased patients showed a lower mean observed/expected lung-to-head ratio (O/E-LHR%) both at diagnosis and before birth, and the liver was herniated more frequently. Moreover, both gestational age and weight were lower, and patch insertion was significantly higher (**Table 2**).

At all times, deceased patients showed higher systolic pulmonary arterial pressure (sPAP) values compared with survivors (sPAP T0: 64.4 ± 17.2 vs. 54.4 ± 5.3 mmHg, $p = 0.016$; sPAP T1: 60.7 ± 10.9 vs. 50.6 ± 16.2 mmHg, $p = 0.022$; sPAP T2: 55.1 ± 17.9 vs. 46.4 ± 17.7 mmHg, $p = 0.163$; sPAP T3: 65.7 ± 16.5 vs. 38.8 ± 13.6 mmHg, $p < 0.001$; **Figure 2A**). They also showed lower mean total, ipsilateral, and contralateral pulmonary areas at birth (total pulmonary area: 8.1 ± 4.6 vs. 15.1 ± 6.9 cm^2, $p < 0.001$; ipsilateral pulmonary area: 1.8 ± 2.6 vs. 5.1 ± 3.4 cm^2, $p < 0.001$; contralateral pulmonary area, 6.2 ± 3.0 vs. 10.0 ± 4.3 cm^2, $p < 0.001$; **Figure 2B**).

TABLE 2 | Comparison between deceased and survived patients.

	Deceased ($n = 28$)	Survived ($n = 49$)	p-value
Prenatal data			
Side of defect—*n* (%)			
- Left CDH	22 (78.6)	39 (79.6)	0.856^
- Right CDH	6 (21.4)	9 (18.4)	
- Bilateral CDH	0 (0.0)	1 (2.0)	
O/E LHR%—mean (*SD*)			
- Initial	28.4 (7.6)	40.1 (13.4)	<0.001*
- Final	42.1 (13.5)	54.2 (15.3)	0.001*
Liver up—*n* (%)	28 (100)	23 (46.9)	<0.001^
Grading CDH—*n* (%)			
- Severe	19 (67.9)	13 (26.5)	<0.001^
- Moderate	7 (25.0)	6 (12.2)	
- Mild	2 (7.1)	30 (61.2)	
FETO—*n* (%)	16 (57.1)	12 (24.5)	0.006*
Postnatal data			
Gestational age (weeks)—mean (*SD*)	35.6 (2.4)	37.2 (1.9)	0.002*
Birth weight (g)—mean (*SD*)	2437 (438)	2920 (591)	<0.001*
Males—*n* (%)	13 (46.4)	30 (61.2)	0.239^
Vaginal delivery—*n* (%)	8 (28.6)	32 (65.3)	0.002^
APGAR 1°min—median (IQR)	4.5 (3–6)	6 (5–8)	0.003°
APGAR 5°min—median (IQR)	7 (6–8)	8 (8–9)	<0.001°
Surgery—*n* (%)	17 (60.7)	49 (100)	<0.001^
Day of surgical repair—median (IQR)	3 (2–4)	2 (2–3.5)	0.603°
Diaphragmatic patch (on operated)—*n* (%)	15 (88.2)	19 (38.8)	0.001^
Abdominal patch (on operated)—*n* (%)	0 (0.0)	1 (2.0)	>0.999^
Mechanical ventilation (days)—median (IQR)	8 (2–23.5)	16 (9–20)	0.036°
Oxygen (days)—median (IQR)	7.5 (2–31)	13 (3–27)	0.426°
Nitric oxide (days)—median (IQR)	8 (2–21)	9 (0–15)	0.380°
Sildenafil (days)—median (IQR)	7 (2–29.5)	0 (0–31)	0.077°
Length of stay (days)—median (IQR)	8 (2–31)	44 (35.5–70.5)	<0.001°

CDH, congenital diaphragmatic hernia; FETO, fetal endoscopic tracheal occlusion; IQR, interquartile range; n, number; O/E LHR, observed/expected lung-to-head ratio; SD, standard deviation.
**Student's T-Test.*
°Mann Whitney U-Test.
^Fisher Exact Test.

At birth, pulmonary area and sPAP were significantly associated: as the three areas increased, sPAP at T0 significantly decreased, as shown by the linear regression model (**Table 3A**).

Following logistic regression analysis with death as the outcome variable, the increase in all radiographic parameters was also significantly related to improved survival in the first year of life (**Table 3B**).

Finally, with increasing sPAP at T0, the risk of death significantly increased as well (**Table 3C**).

The receiver operating characteristic (ROC) curve analysis showed that the total pulmonary area had an area under the curve (AUC) of 0.808, and a cut-off of 10.87 cm^2 predicted survival with 77.6% sensitivity and 75% specificity (**Figure 3A1**). The ipsilateral pulmonary area had an AUC of 0.772, and a cut-off of 2.08 cm^2 predicted survival with 81.6% sensitivity and 68% specificity (**Figure 3A2**). The contralateral pulmonary area had an AUC of 0.775, and a cut-off of 7.3 cm^2 predicted survival with 75% sensitivity and 68% specificity (**Figure 3A3**).

We finally performed aROC curve analysis using the O/E LHR at diagnosis. The total pulmonary area had an area AUC of 0.765, and a cut-off value of 31.7% showed 74% sensitivity and 74% specificity in predicting survival (**Figure 3B**).

Radiographic Pulmonary Area and Hernia Recurrence

Survivors at the end of the first year of life were divided into two groups based on hernia recurrence: recurrence ($n = 10$) and non-recurrence ($n = 39$; **Table 4**).

The recurrence group mainly included severe-moderate forms (80 vs. 28.2%), while most non-recurrence patients were mild (20 vs. 71.8%). Although the mean initial O/E-LHR% was not significantly different, the mean final O/E-LHR% was lower in the recurrence group (44.4 vs. 56.9%, $p = 0.029$). Even though diaphragmatic patching was higher in the recurrence group, this difference was not significant. Recurrence patients required longer intensive care (**Table 4**).

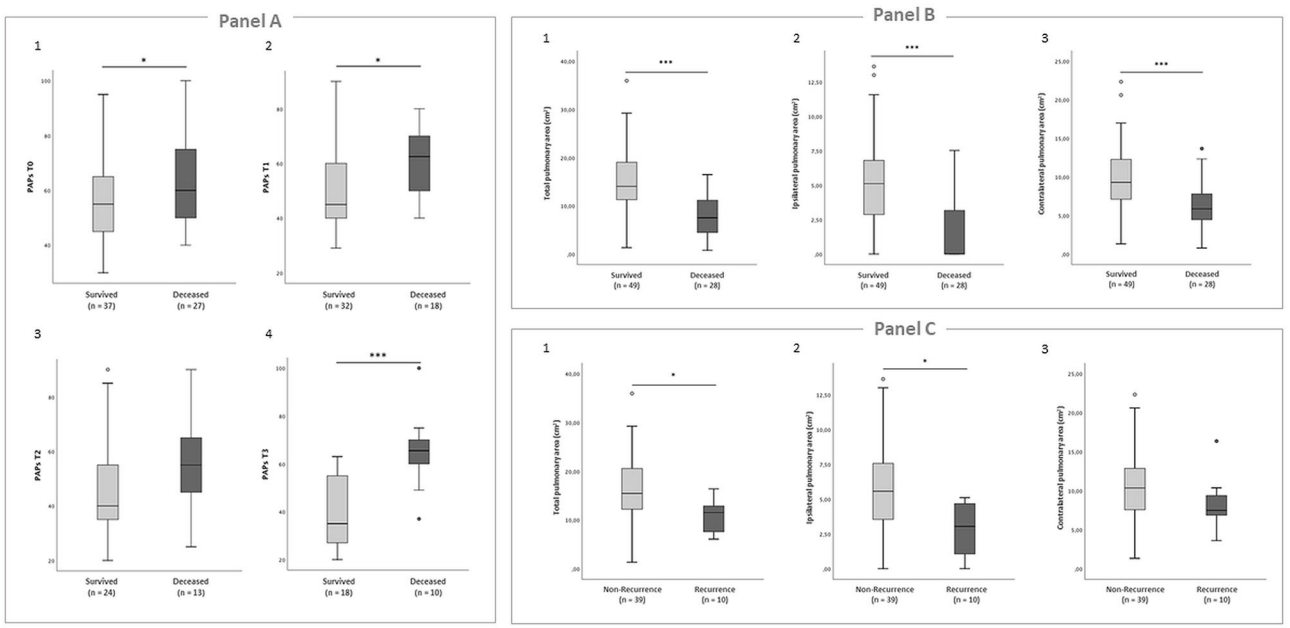

FIGURE 2 | (A) Boxplots showing the comparison of sPAP values between survived and deceased patients. Student's *t*-test was performed to compare the two groups. (1) sPAP T0, $p = 0.016$; (2) sPAP T1, $p = 0.022$; (3) sPAP T2, $p = 0.163$; (4) sPAP T3, $p < 0.001$. **(B)** Boxplots showing the comparison of radiographic lung area on the first day of life between survived and deceased patients. Student's *t*-test was performed to compare the two groups. (1) Total pulmonary area, $p < 0.001$; (2) ipsilateral pulmonary area, $p < 0.001$; (3) contralateral pulmonary area, $p < 0.001$. **(C)** Boxplots showing the comparison of radiographic lung area on the first day of life between non-recurrence and recurrence patients. Student's *t*-test was performed to compare the two groups. (1) Total pulmonary area, $p = 0.034$; (2) ipsilateral pulmonary area, $p = 0.011$; (3) contralateral pulmonary area, $p = 0.164$.

The mean total and ipsilateral pulmonary area were significantly lower in the recurrence compared with non-recurrence group (total pulmonary area: 11.0 ± 3.2 vs. 16.2 ± 7.2 cm^2, $p = 0.034$; ipsilateral pulmonary area: 2.7 ± 2 vs. 5.7 ± 3.4 cm^2, $p = 0.011$), while the mean contralateral area was not significantly different (8.3 ± 3.3 vs. 10.5 ± 4.5 cm^2, $p = 0.164$; **Figure 2C**).

The logistic regression model showed that as the total and ipsilateral areas increased, CDH recurrence significantly decreased (**Table 3D**).

The ROC curve analysis showed that the total pulmonary area had an AUC of 0.759, and a cut-off of 13.07 cm^2 predicted a 1-year follow-up free of hernia recurrence with 71.8% sensitivity and 80% specificity (**Figure 3C1**). The ipsilateral pulmonary area had an AUC of 0.790, and a cut-off of 3.75 cm^2 had 74.4% sensitivity and 60% specificity (**Figure 3C2**).

Comparison Between FETO and Non-FETO Patients

Mild cases have been excluded from the non-FETO population to achieve a more homogeneous CDH population of moderate-severe cases, either treated *in utero* or expectantly managed. The new population was constituted 45 patients, divided into 28 FETO (100% severe) and 17 non-FETO (76.5% moderate and 23.5% severe) (**Table 5**).

FETO group was more severely affected, as showed by lower mean O/E-LHR% at diagnosis and a higher liver herniation rate. However, the mean O/E-LHR% before birth was higher.

No differences were found in the mean total and contralateral pulmonary area, while the mean ipsilateral pulmonary area was significantly increased in the FETO group.

Mean sPAP values, length of pharmacological treatments, and mechanical ventilation were not significantly different. Despite lower gestational age at birth, no significant difference in mortality rate was observed (FETO 57.1% vs. non-FETO 58.8%, $p > 0.999$). The recurrence rate among survivors did not reach statistical significance ($p = 0.074$).

DISCUSSION

Our study showed an association between radiographic lung area, sPAP values, and death, confirming pulmonary hypoplasia and pulmonary hypertension as the two most important determinants of mortality (25, 26, 36). Among survivors, lung area was also associated with hernia recurrence. As previously reported, our findings suggest a possible role of the radiographic lung area assessment as an easy, non-invasive, and reproducible tool in the early prediction of mortality and morbidity among patients with CDH (17, 18).

In our cohort, lower O/E-LHR% in the deceased group indicated a more severe fetal lung impairment, which was then reflected in smaller pulmonary areas at birth. Consequently, lung

TABLE 3 | Radiographic lung area and outcome.

A	**Radiographic pulmonary area**		**sPAP at T0**	
	Area (cm^2)	**Estimate**	**95%CI**	***p*-value**
	Total	−0.85	−1.44, −0.25	0.006
	Ispilateral	−1.84	−3.06, −0.62	0.004
	Contralateral	−1.09	−2.08, −0.09	0.032
B	**Radiographic pulmonary area**		**Death**	
	Area (cm^2)	**OR**	**95%CI**	***p*-value**
	Total	0.78	0.69, 0.89	<0.001
	Ipsilateral	0.57	0.43, 0.76	<0.001
	Contralateral	0.76	0.63, 0.91	0.003
C	**Pulmonary hypertension**		**Death**	
	sPAP (mmHg)	**OR**	**95%CI**	***p*-value**
	T0	1.04	1.00, 1.07	0.034
D	**Radiographic pulmonary area**		**Recurrence**	
	Area (cm^2)	**OR**	**95%CI**	***p*-value**
	Total	0.86	0.75, 1.00	0.042
	Ipsilateral	0.71	0.53, 0.95	0.022
	Contralateral	0.86	0.71, 1.05	0.148

Results are corrected for gestational age. sPAP, Systolic Pulmonary Arterial Pressure.

area and death were inversely related: 1 cm^2 of rising in the ipsilateral area was associated with a 43% reduction in mortality, while variations in the total and contralateral area determined a reduction of 22 and 24%, respectively.

Wide defects have been previously associated with worse survival and pulmonary hypertension, suggesting that small lung size depicts the link between these two elements (6). Similarly, in our cohort, deceased infants were characterized by persistently higher sPAP values than survivors. In particular, sPAP values at birth showed a decreasing trend by 1.84 mmHg, with each 1 cm^2 increase in the ipsilateral area.

Our findings were consistent with previous literature (13, 16, 18). A significantly lower CRTA was reported in newborns with CDH who died compared with survivors, and a CRTA >12.99 cm^2 was found to predict survival to discharge from NICU better than LHR at diagnosis, with 85% sensitivity and 73% specificity (18). In our study, we considered the O/E LHR% at diagnosis instead of the absolute ratio, but similarly, the lung area performed better in predicting mortality.

After surgical repair, persistently elevated pulmonary pressure carried the highest mortality risk, with a 16% increase in death risk for each sPAP unitary increment. Several studies have correlated the severity of PH with mortality. Dillon et al. evaluated mortality in a cohort of CDH patients and reported that all those with supra-systemic sPAP died (26). Coughlin et al. reported that patients with higher pulmonary pressure at 1 month had a higher incidence of post-operative complications and worse survival, and persistently severe PH at 1 month was associated with increased mortality (6). Similarly, looking at our results, we could assume that the most critical factor might not be the absolute value of sPAP or the presence of PH in the first hours after birth, rather its persistence over time (6).

We also observed a significant association between preoperative radiographic measurements and hernia recurrence among survivors during the first year of life. The overall recurrence rate of 20.4% in our cohort was in line with the literature reports (19, 24, 37). In particular, the recurrence rate was higher in those patients with lower final O/E LHR%, prolonged invasive respiratory support, and need for intensive care. Similarly, Al-Iede et al. found a longer duration of mechanical ventilation and hospitalization in children with recurrence (21). Notably, these patients showed a significantly lower mean total pulmonary area at birth than non-recurrence, mainly due to a significantly lower ipsilateral pulmonary area.

As a consequence, we respectively, observed a 14 and 29% reduction in recurrence risk in our cohort per unit increment of the total and ipsilateral area. The total radiographic area had the best specificity in discriminating those patients at risk of recurrence, while the ipsilateral area showed better sensitivity. Taken together, the lower ipsilateral area and O/E-LHR% reflected the presence of a large diaphragmatic defect as the cause of poor lung development, indirectly confirming defect size as the leading risk factor for hernia recurrence (19–22, 24). In other words, we speculate that recurrence patients were somehow "predisposed" to this complication since birth and could have been identified early in the postnatal course. The recurrence group's high patching rate suggested the presence of a wide defect, although this difference did not reach statistical significance. We cannot deduce any specific contribution of the patch in determining the recurrence risk due to the low sample size.

We observed that tracheal occlusion improved lung development and outcome through the descriptive comparison between FETO and non-FETO patients' characteristics. Since prenatal treatment is reserved for severe cases of CDH, the FETO group included only patients at one end of the spectrum of disease severity (2). Nevertheless, final O/E-LHR% dramatically improved after the procedure, and the ipsilateral lung area at birth was even significantly better so that the total pulmonary area did not differ between the two groups. Likewise, Dassios et al. observed that patients previously submitted to the FETO procedure had a CRTA comparable with untreated patients with a similar mortality rate, reflecting a lung catch-up growth favored by the prenatal procedure (18).

In our cohort, the non-FETO group, which was primarily constituted by moderate cases, showed a 41.2% survival rate, in line with what is generally expected for this category of CDH (2, 38, 39). As observed by Doneè et al., tracheal occlusion allowed improved outcomes in the operative group, similar to a moderate population expectantly managed (40). Finally, the recurrence risk was not significantly different between the two populations, despite higher patching in the FETO group, as previously observed by Ali et al. (41). Although patch repair is a leading risk factor, the low recurrence rate suggests other factors besides patch use as possible re-herniation determinants (20, 42). Tsai et al. reported a non-significant difference in recurrence rate between patching and primary repair, despite a higher disease severity in the first group (20). Jawaid et al. reported a low incidence of recurrence in patients in which Gore-Tex® patch was inserted

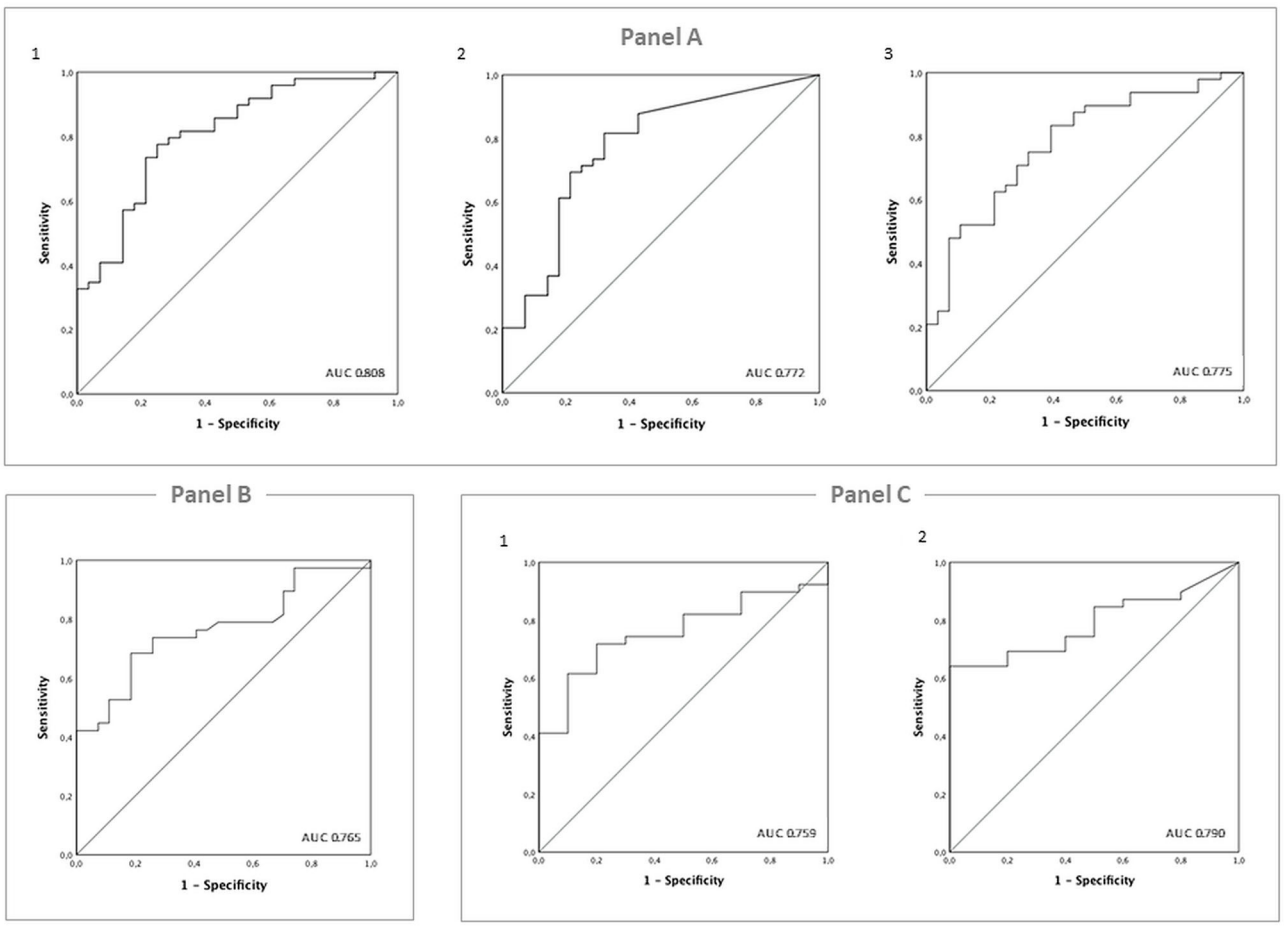

FIGURE 3 | (A) ROC analysis and estimation of the corresponding area under the curve (AUC) for the radiographic pulmonary area's performance in predicting survival to 1 year of life. (1) Total pulmonary area; (2) ipsilateral pulmonary area; (3) contralateral pulmonary area. **(B)** ROC analysis and estimation of the corresponding area under the curve (AUC) for the O/E LHR% at diagnosis performance in predicting hernia recurrence in the first year of life. **(C)** ROC analysis and estimation of the corresponding area under the curve (AUC) for the radiographic performance of the pulmonary area in predicting hernia recurrence in the first year of life. (1) Total pulmonary area; (2) ipsilateral pulmonary area.

(42). Although we cannot conclude on the patch's contribution to re-herniation, we can observe that lower radiographic area at birth could influence the risk of this complication and speculate that lung catch-up growth in FETO patients could confer the same recurrence risk as the untreated counterparts, which needs to be confirmed with further data (19, 21, 22, 24).

To the best of our knowledge, our study seems to be the first to evaluate the association between radiographic lung area and two important outcomes affecting newborns with CDH: pulmonary hypertension and hernia recurrence.

The radiographic measurement is easy, rapid, and can be performed soon after birth on the chest X-ray routinely performed at NICU admission. It would contribute to the early identification of infants at greater risk of developing higher sPAP values in the immediate postnatal period and a higher likelihood of long-term hernia recurrence and higher mortality. For example, the combined serial evaluation of lung area and sPAP over time could help to define trajectories related to the risk of persistently elevated sPAP and chronic pulmonary hypertension. Similarly, the preoperative radiographic assessment could help identify a subgroup of patients at higher risk of recurrence, directing them toward a tailored surgical follow-up.

The ipsilateral and contralateral areas were considered separately, evaluating the impact of hernia on each lung. We demonstrated that the ipsilateral area, which is more seriously affected by visceral herniation, has the most significant influence on patient outcomes.

Finally, focusing on FETO patients, we confirm the positive effects of the fetal procedure on lung catch-up growth and patient outcome.

Patients from our cohort showed a broad spectrum of disease severity, including infants requiring fetal surgery and ECMO support, and the standardization of treatment according to international guidelines guarantees uniformity of care.

A certain technical difficulty in tracing the lung perimeter in severe forms must be underlined as a limitation of the study. We arbitrarily decided to consider only those parts of the radiograms where a lung plot was present, corresponding to those regions

TABLE 4 | Comparison between recurrence and non-recurrence hernia patients.

	Recurrence ($n = 10$)	Non-recurrence ($n = 39$)	p-value
Prenatal data			
Side of defect—*n* (%)			>0.999^
- Left CDH	8 (80.0)	31 (79.5)	
- Right CDH	2 (20.0)	7 (17.9)	
- Bilateral CDH	0 (0.0)	1 (2.6)	
O/E LHR%—mean (*SD*)			
- Initial	34.6 (8.2)	42.1 (14.4)	0.132*
- Final	44.4 (14.6)	56.9 (14.6)	0.029*
Liver UP—*n* (%)	5 (50)	18 (46.2)	>0.999^
Grading CDH—*n* (%)			
- Severe	4 (40.0)	9 (23.1)	0.002^
- Moderate	4 (40.0)	2 (5.1)	
- Mild	2 (20.0)	28 (71.8)	
FETO—*n* (%)	3 (30.0)	9 (23.1)	0.690^
Postnatal data			
Gestational age (weeks)—mean (*SD*)	37.5 (1.5)	37.1 (2.0)	0.563*
Birthweight (g)—mean (*SD*)	2808 (412)	2949 (630)	0.506*
Day of surgical repair—median (IQR)	3 (2.75–4.25)	2 (2–3)	0.066°
Diaphragmatic patch—*n* (%)	6 (60.0)	13 (33.3)	0.156^
Abdominal patch—*n* (%)	0 (0.0)	1 (2.6)	>0.999^
Mechanical ventilation (days)—median (IQR)	20.5 (15.25–26)	12 (8–18)	0.013°
Length of stay (days)—median (IQR)	55 (43–111.75)	42 (33–66)	0.028°

CDH, congenital diaphragmatic hernia; FETO, fetal endoscopic tracheal occlusion; IQR, interquartile range; O/E LHR, observed/expected lung-to-head ratio; SD, standard deviation.
**Student's T-Test.*
°*Mann Whitney U-Test.*
^*Fisher Exact Test.*

effectively recruited and ventilated. However, the interference of mechanical compression exerted by the herniated organs plays a considerable role.

This methodological decision could constitute a bias leading to underestimating the lung dimensions since atelectasis areas had been excluded from the measurement. Therefore, after mechanical compression has been removed, the effective lung area evaluation could reliably define lung hypoplasia and associated outcome. For example, Dimitriou et al. calculated the difference between the pre- and post-operative radiographic measurements, showing that post-operative improvement was higher in patients with a good outcome. They concluded that poor prognosis was correlated to low post-operative rather than low preoperative values, which was probably more related to mechanical compression than lung hypoplasia (16). Therefore, the radiographic assessment of post-operative lung areas and the relative increase from preoperative values should be included in further analysis.

The neonatal ECMO Center was activated in 2016, with only three patients undergoing extracorporeal support during the study period. This could have had an impact on survival chances. In addition, team training and expertise plays a major role in favorable ECMO outcome. We expect a survival improvement in the very recent years, which we would like to confirm with additional analysis (43–45).

Another significant weakness is related to the retrospective design of the study, which limited the sample size. Some missing data regarding sPAP estimation could not be integrated with further hemodynamic assessments. Therefore, only an exploratory secondary analysis could be performed. Finally, we recognize that several factors could influence pulmonary vascular resistance and mortality throughout the hospital stay, such as pharmacological treatments, infections, patency of the ductus arteriosus, or surgery timing. Similarly, radiographic lung area could be influenced by factors such as quality of image, ventilator settings, under- or overinflation, which can then influence vascular resistance as well. The contribution of these factors cannot be completely assessed with single imaging performed at birth, which reflects the patient's conditions in a defining moment. Although the highest lung area can be considered a good approximation, it might not reflect the patient best clinical condition, and these factors should be taken into account in further analysis on a larger cohort. In addition, it would be of great importance to match lung area and sPAP values at T1, T2, and T3, to clarify if the association is still confirmed over time and define possible trajectories.

CONCLUSIONS

The radiographic pulmonary area on the first day of life reflects impaired lung development during fetal life and the extent of the diaphragmatic defect in CDH patients. Lower lung areas are associated with higher sPAP values at birth, death, and hernia recurrence. Further studies are needed to consolidate these results and define the possible role of the radiographic

TABLE 5 | Comparison between FETO and non-FETO patients.

	FETO ($n = 28$)	Non-FETO, excluded mild ($n = 17$)	*p*-value
Prenatal data			
Side of defect—*n* (%)			
- Left CDH	19 (67.9)	15 (88.2)	0.341^
- Right CDH	8 (28.6)	2 (11.8)	
- Bilateral CDH	1 (3.6)	0 (0.0)	
Liver UP—*n* (%)	28 (100)	13 (76.5)	0.016^
O/E LHR%—mea *n* (SD)			
- Initial	25.4 (5.6)	35.1 (7.9)	<0.001*
- Final	51.8 (15.4)	33.8 (7.1)	<0.001*
Grading CDH—*n* (%)			
- Severe	28 (100)	4 (23.5)	<0.001^
- Moderate	0 (0.0)	13 (76.5)	
Postnatal data			
Gestational age (weeks)—mean (SD)	35 (2.4)	37.1 (1.7)	0.003*
Birthweight (g)—mean (*SD*)	2436 (511)	2517 (389)	0.576*
Surgery—*n* (%)	22 (78.6)	12 (70.6)	0.722^
Day of surgical repair—median (IQR)	2.5 (2–3)	3.5 (2.25–5)	0.040°
Diaphragmatic patch (on operated)—*n* (%)	18 (81.8)	8 (66.7)	0.410^
Abdominal patch (on operated)—*n* (%)	1 (4.5)	0 (0.0)	>0.999^
sPAP T0 (mmHg)—mean (*SD*)	59.4 (16.4)	63.2 (18.6)	0.513*
sPAP T1 (mmHg)—mean (*SD*)	55.2 (13.0)	63.1 (13.6)	0.136*
sPAP T2 (mmHg)—mean (*SD*)	48.3 (15.2)	53.5 (18.7)	0.459*
sPAP T3 (mmHg)—mean (*SD*)	54.6 (20.3)	42.9 (20.7)	0.228*
Mechanical ventilation (days)—median (IQR)	16 (9–25.5)	15 (5–23)	0.582°
Oxygen (days)—median (IQR)	16 (4.75–34.75)	13 (2.5–45)	0.761°
Nitric oxide (days)—median (IQR)	11.5 (6–22)	9 (2.5–17)	0.337°
Sildenafil (days)—median (IQR)	9 (1–74.25)	6 (0–38)	0.334°
Length of stay (days)—median (IQR)	41 (9–94.5)	39 (5–80.5)	0.512°
Deceased—*n* (%)	16 (57.1)	10 (58.8)	>0.999^
Recurrence (on survivors)—*n* (%)	3 (25.0)	5 (71.4)	0.074^
Radiographic pulmonary area			
Total pulmonary area (cm^2)—mean (*SD*)	10.5 (6.1)	8.9 (4.7)	0.362*
Ipsilateral pulmonary area (cm^2)—mean (*SD*)	3.5 (3.2)	1.3 (2.0)	0.015*
Contralateral pulmonary area (cm^2)—mean (*SD*)	6.9 (3.5)	7.6 (3.8)	0.523*

CDH, congenital diaphragmatic hernia; FETO, fetal endoscopic tracheal occlusion; IQR, interquartile range; n, number; O/E LHR, observed/expected lung-to-head ratio; SD, standard deviation; sPAP, Systolic Pulmonary Arterial Pressure.

**Student's T-Test.*

°Mann Whitney U-Test.

^Fisher Exact Test.

lung area for early risk assessment, monitoring, and outcome prediction in newborns with CDH.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: ClinicalTrials.gov n° NCT04396028.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Milan Area 2, Italy. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

IA, GC, GR, SGa, SGh, VC, GB, NPes, and FMo contributed to the study's conception and design. IA, GR, GC, VC, SGa, SGh, and FMa wrote the first draft of the manuscript. IA, NPes, and GC calculated the sample size. IA and NPes performed the statistical analysis. IA and IB assessed radiographic pulmonary areas. IB, NPer, IF, FMa, AC, MC, and FMo provided extensive critical revision. All authors contributed to the manuscript's critical revision and read and approved the submitted version.

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REFERENCES

1. Grover TR, Murthy K, Brozanski B, Gien J, Rintoul N, Keene S, et al. Short-term outcomes and medical and surgical interventions in infants with congenital diaphragmatic hernia. *Am J Perinatol.* (2015) 32:1038–44. doi: 10.1055/s-0035-1548729
2. Deprest J, Brady P, Nicolaides K, Benachi A, Berg C, Vermeesch J, et al. Prenatal management of the fetus with isolated congenital diaphragmatic hernia in the era of the TOTAL trial. *Semin Fetal Neonatal Med.* (2014) 19:338–48. doi: 10.1016/j.siny.2014.09.006
3. Steinhorn RH, Abman SH. 52 - Persistent pulmonary hypertension. In: Gleason CA, Juul SE, editors. *Avery's Diseases of the Newborn.* 10th Ed. Philadelphia, PA: Elsevier (2018). p. 768–78.e3. doi: 10.1016/B978-0-323-40139-5.00052-8
4. Pierro M, Thebaud B. Understanding and treating pulmonary hypertension in congenital diaphragmatic hernia. *Semin Fetal Neonatal Med.* (2014) 19:357–63. doi: 10.1016/j.siny.2014.09.008
5. Snoek KG, Greenough A, Van Rosmalen J, Capolupo I, Schaible T, Ali K, et al. Congenital diaphragmatic hernia: 10-year evaluation of survival, extracorporeal membrane oxygenation, and foetoscopic endotracheal occlusion in four high-volume centres. *Neonatology.* (2018) 113:63–8. doi: 10.1159/000480451
6. Coughlin MA, Werner NL, Gajarski R, Gadepalli S, Hirschl R, Barks J, et al. Prenatally diagnosed severe CDH: mortality and morbidity remain high. *J Pediatr Surg.* (2016) 51:1091–5. doi: 10.1016/j.jpedsurg.2015.10.082
7. Bagolan P, Morini F. Long-term follow up of infants with congenital diaphragmatic hernia. *Semin Pediatr Surg.* (2007) 16:134–44. doi: 10.1053/j.sempedsurg.2007.01.009
8. American Academy of Pediatrics Section on Surgery; American Academy of Pediatrics Committee on Fetus and Newborn, Lally KP, Engle W. Postdischarge follow-up of infants with congenital diaphragmatic hernia. *Pediatrics.* (2008) 121:627–32. doi: 10.1542/peds.2007-3282
9. Rocha G, Azevedo I, Pinto JC, Guimarães H. Follow-up of the survivors of congenital diaphragmatic hernia. *Early Hum Dev.* (2012) 88:255–8. doi: 10.1016/j.earlhumdev.2011.08.025
10. Chen C, Jeruss S, Chapman JS, Terrin N, Tighiouart H, Glassman E, et al. Long-term functional impact of congenital diaphragmatic hernia repair on children. *J Pediatr Surg.* (2007) 42:657–65. doi: 10.1016/j.jpedsurg.2006.12.013
11. Patel N, Kipfmueller F. Cardiac dysfunction in congenital diaphragmatic hernia: pathophysiology, clinical assessment, and management. *Semin Pediatr Surg.* (2017) 26:154–8. doi: 10.1053/j.sempedsurg.2017.04.001
12. Rafat N, Schaible T. Extracorporeal membrane oxygenation in congenital diaphragmatic hernia. *Front Pediatr.* (2019) 7:336. doi: 10.3389/fped.2019.00336
13. Dassios T, Curley A, Krokidis M, Morley C, Ross-Russell R. Correlation of radiographic thoracic area and oxygenation impairment in bronchopulmonary dysplasia. *Respir Physiol Neurobiol.* (2016) 220:40–5. doi: 10.1016/j.resp.2015.09.009
14. May C, Prendergast M, Salman S, Rafferty GF, Greenough A. Chest radiograph thoracic areas and lung volumes in infants developing bronchopulmonary dysplasia. *Pediatr Pulmonol.* (2009) 44:80–5. doi: 10.1002/ppul.20952
15. Dimitriou G, Greenough A, Kavvadia V, Shute M, Karani J. A radiographic method for assessing lung area in neonates. *Br J Radiol.* (1999) 72:335–8. doi: 10.1259/bjr.72.856.10474492
16. Dimitriou G, Greenough A, Davenport M, Nicolaides K. Prediction of outcome by computer-assisted analysis of lung area on the chest radiograph of infants with congenital diaphragmatic hernia. *J Pediatr Surg.* (2000) 35:489–93. doi: 10.1016/S0022-3468(00)90219-7
17. Amodeo I, Raffaeli G, Pesenti N, Macchini F, Condò V, Borzani I, et al. The NeoAPACHE study protocol i: assessment of the radiographic pulmonary area and long-term respiratory function in newborns with congenital diaphragmatic hernia. *Front Pediatr.* (2020) 8:581809. doi: 10.3389/fped.2020.581809
18. Dassios T, Ali K, Makin E, Bhat R, Krokidis M, Greenough A. Prediction of mortality in newborn infants with severe congenital diaphragmatic hernia using the chest radiographic thoracic area. *Pediatr Crit Care Med Soc Crit Care Med.* (2019) 20:534–9. doi: 10.1097/PCC.0000000000001912
19. Putnam LR, Gupta V, Tsao K, Davis CF, Lally PA, Lally KP, et al. Factors associated with early recurrence after congenital diaphragmatic hernia repair. *J Pediatr Surg.* (2017) 52:928–32. doi: 10.1016/j.jpedsurg.2017.03.011
20. Tsai J, Sulkowski J, Adzick NS, Hedrick HL, Flake AW. Patch repair for congenital diaphragmatic hernia: is it really a problem? *J Pediatr Surg.* (2012) 47:637–41. doi: 10.1016/j.jpedsurg.2011.11.054
21. Al-Iede MM, Karpelowsky J, Fitzgerald DA. Recurrent diaphragmatic hernia: modifiable and non-modifiable risk factors. *Pediatr Pulmonol.* (2016) 51:394–401. doi: 10.1002/ppul.23305
22. Fisher JC, Haley MJ, Ruiz-Elizalde A, Stolar CJ, Arkovitz MS. Multivariate model for predicting recurrence in congenital diaphragmatic hernia. *J Pediatr Surg.* (2009) 44:1173–80. doi: 10.1016/j.jpedsurg.2009.02.043
23. Moss RL, Chen CM, Harrison MR. Prosthetic patch durability in congenital diaphragmatic hernia: a long-term follow-up study. *J Pediatr Surg.* (2001) 36:152–4. doi: 10.1053/jpsu.2001.20037
24. Nagata K, Usui N, Terui K, Takayasu H, Goishi K, Hayakawa M, et al. Risk factors for the recurrence of the congenital diaphragmatic hernia—report from the long-term follow-up study of Japanese CDH study group. *Eur J Pediatr Surg.* (2015) 25:9–14. doi: 10.1055/s-0034-1395486
25. Leeuwen L, Fitzgerald DA. Congenital diaphragmatic hernia. *J Paediatr Child Health.* (2014) 50:667–73. doi: 10.1111/jpc.12508
26. Dillon PW, Cilley RE, Mauger D, Zachary C, Meier A. The relationship of pulmonary artery pressure and survival in congenital diaphragmatic hernia. *J Pediatr Surg.* (2004) 39:307–12. doi: 10.1016/j.jpedsurg.2003.11.010
27. Kitagawa M, Hislop A, Boyden E, Reid L. Lung hypoplasia in congenital diaphragmatic hernia a quantitative study of airway, artery, and alveolar development. *Br J Surg.* (1971) 58:342–6. doi: 10.1002/bjs.1800580507
28. Russo FM, De Coppi P, Allegaert K, Toelen J, van der Veeken L, Attilakos G, et al. Current and future antenatal management of isolated congenital diaphragmatic hernia. *Semin Fetal Neonatal Med.* (2017) 22:383–90. doi: 10.1016/j.siny.2017.11.002
29. Van der Veeken L, Russo FM, De Catte L, Gratacos E, Benachi A, Ville Y, et al. Fetoscopic endoluminal tracheal occlusion and reestablishment of fetal airways for congenital diaphragmatic hernia. *Gynecol Surg.* (2018) 15:9. doi: 10.1186/s10397-018-1041-9
30. Snoek KG, Reiss IK, Greenough A, Capolupo I, Urlesberger B, Wessel L, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH EURO consortium consensus-2015 update. *Neonatology.* (2016) 110:66–74. doi: 10.1159/000444210
31. Ito M, Terui K, Nagata K, Yamoto M, Shiraishi M, Okuyama H, et al. Clinical guidelines for the treatment of congenital diaphragmatic hernia. *Pediatr Int.* (2021) 63:371–90. doi: 10.1111/ped.14473
32. Boloker J, Bateman DA, Wung JT, Stolar CJ. Congenital diaphragmatic hernia in 120 infants treated consecutively with permissive hypercapnea/spontaneous respiration/elective repair. *J Pediatr Surg.* (2002) 37:357–66. doi: 10.1053/jpsu.2002.30834
33. Rozmiarek AJ, Qureshi FG, Cassidy L, Ford HR, Hackam DJ. Factors influencing survival in newborns with congenital diaphragmatic hernia: the relative role of timing of surgery. *J Pediatr Surg.* (2004) 39:821–4. doi: 10.1016/j.jpedsurg.2004.02.010
34. Tsao K, Lally KP. The Congenital Diaphragmatic Hernia Study Group: a voluntary international registry. *Semin Pediatr Surg.* (2008) 17:90–7. doi: 10.1053/j.sempedsurg.2008.02.004

35. Hollinger LE, Buchmiller TL. Long term follow-up in congenital diaphragmatic hernia. *Semin Perinatol.* (2020) 44:151171. doi: 10.1053/j.semperi.2019.07.010
36. Harting MT. Congenital diaphragmatic hernia-associated pulmonary hypertension. *Semin Pediatr Surg.* (2017) 26:147–53. doi: 10.1053/j.sempedsurg.2017.04.008
37. Rowe DH, Stolar CJ. Recurrent diaphragmatic hernia. *Semin Pediatr Surg.* (2003) 12:107–9. doi: 10.1016/S1055-8586(02)00020-3
38. Jani J, Nicolaides K, Keller R, Benachi A, Peralta C, Favre R, et al. Observed to expected lung area to head circumference ratio in the prediction of survival in fetuses with isolated diaphragmatic hernia. *Ultrasound Obstetr Gynecol.* (2007) 30:67–71. doi: 10.1002/uog.4052
39. Jani J, Nicolaides KH, Gratacos E, Valencia C, Doné E, Martinez JM, et al. Severe diaphragmatic hernia treated by fetal endoscopic tracheal occlusion. *Ultrasound Obstetr Gynecol.* (2009) 34:304–10. doi: 10.1002/uog.6450
40. Doné E, Gratacos E, Nicolaides K, Allegaert K, Valencia C, Castañon M, et al. Predictors of neonatal morbidity in fetuses with severe isolated congenital diaphragmatic hernia undergoing fetoscopic tracheal occlusion. *Ultrasound Obstetr Gynecol.* (2013) 42:77–83. doi: 10.1002/uog.12445
41. Ali K, Dassios T, Khaliq SA, Williams EE, Tamura K, Davenport M, et al. Outcomes of infants with congenital diaphragmatic hernia by side of defect in the FETO era. *Pediatr Surg Int.* (2019) 35:743–7. doi: 10.1007/s00383-019-04484-3
42. Jawaid W, Qasem E, Jones M, Shaw N, Losty P. Outcomes following prosthetic patch repair in newborns with congenital diaphragmatic hernia. *Br J Surg.* (2013) 100:1833–7. doi: 10.1002/bjs.9306
43. Raffaeli G, Ghirardello S, Vanzati M, Baracetti C, Canesi F, Conigliaro F, et al. Start a neonatal extracorporeal membrane oxygenation program: a multistep team training. *Front Pediatr.* (2018) 6:151. doi: 10.3389/fped.2018.00151
44. Wild KT, Rintoul N, Kattan J, Gray B, Keene S, Best D, et al. Extracorporeal Life Support Organization (ELSO): guidelines for neonatal respiratory failure. *Asaio Journal.* (2020) 66:463–70.
45. Macchini F, Di Cesare A, Morandi A, Ichino M, Raffaeli G, Conigliaro F, et al. Surgical expertise in neonatal extracorporeal membrane oxygenation (ECMO): a single center experience. *Front Pediatr.* (2019) 7:398. doi: 10.3389/fped.2019.00398

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Persisting Motor Function Problems in School-Aged Survivors of Congenital Diaphragmatic Hernia

*Sophie de Munck[1], Monique H. M. van der Cammen-van Zijp[1], Tabitha P. L. Zanen-van den Adel[2], René M. H. Wijnen[1], Suzan C. M. Cochius-den Otter[1], Neeltje E. M. van Haren[3], Saskia J. Gischler[1], Joost van Rosmalen[4,5] and Hanneke IJsselstijn[1]**

[1] Department of Pediatric Surgery and Pediatric Intensive Care, Erasmus MC Sophia Children's Hospital, Rotterdam, Netherlands, [2] Department of Orthopedics, Section of Physical Therapy, Erasmus MC Sophia Children's Hospital, Rotterdam, Netherlands, [3] Department of Child and Adolescent Psychiatry and Psychology, Erasmus MC Sophia Children's Hospital, Rotterdam, Netherlands, [4] Department of Biostatistics, Erasmus MC, Rotterdam, Netherlands, [5] Department of Epidemiology, Erasmus MC, Rotterdam, Netherlands

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***Correspondence:**
Hanneke IJsselstijn
h.ijsselstijn@erasmusmc.nl

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Background and Objectives: Children born with congenital diaphragmatic hernia (CDH) and treated with extracorporeal membrane oxygenation (ECMO), are at risk for motor function impairment during childhood. We hypothesized that all children born with CDH are at risk for persistent motor function impairment, irrespective of ECMO-treatment. We longitudinally assessed these children's motor function.

Methods: Children with CDH with and without ECMO-treatment, born 1999–2007, who joined our structural prospective follow-up program were assessed with the Movement Assessment Battery for Children (M-ABC) at 5, 8, 12 years. Z-scores were used in a general linear model for longitudinal analysis.

Results: We included 55 children, of whom 25 had been treated with ECMO. Forty-three (78%) were evaluated at three ages. Estimated mean (95% CI) z-scores from the general linear model were −0.67 (−0.96 to −0.39) at 5 years of age, −0.35 (−0.65 to −0.05) at 8 years, and −0.46 (−0.76 to −0.17) at 12 years. The 5- and 8-years scores differed significantly ($p = 0.02$). Motor development was significantly below the norm in non-ECMO treated patients at five years; −0.44 (−0.83 to −0.05), and at all ages in the ECMO-treated-patients: −0.90 (−1.32 to −0.49), −0.45 (−0.90 to −0.02) and −0.75 (−1.2 to −0.34) at 5, 8, and 12 years, respectively. Length of hospital stay was negatively associated with estimated total z-score M-ABC ($p = 0.004$ multivariate analysis).

Conclusion: School-age children born with CDH are at risk for motor function impairment, which persists in those who received ECMO-treatment. Especially for them long-term follow up is recommended.

Keywords: congenital diaphragmatic hernia, motor function, development, critical illness, follow-up

INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a rare anomaly with a prevalence of 2.3 per 10,000 births (1). This anomaly brings along a broad spectrum of problems, of which lung hypoplasia and pulmonary hypertension contribute considerably to the mortality rate of ∼28% (2). Over the last decade, improved treatment of CDH has increased the survival rates as well as the prevalence of long-term morbidity (1, 3). The latter may last into adolescence and beyond, affecting several domains of development (4, 5). Among all domains, motor impairment is frequently reported, and gross motor function deficits in particular (3). This is of concern, given that motor impairments may affect the child's life on multiple levels, such as not being fully able to participate in sports and lagging behind one's peers. The underlying cause of these impairments remains unknown; yet, a number of determinants have been suggested, disease- as well as treatment-related (6–8). Nevertheless, the identification of specific risk factors might help to develop a tool for risk stratification. Survivors of CDH are already at risk for motor delay in the first years of life (9), although contradictory results on motor development in toddlers born with CDH have been published (10, 11). Aged 5 and 8 years, children born with CDH have been found to be at risk for impaired motor function, regardless of having received extracorporeal membrane oxygenation (ECMO) treatment (6, 12). In a longitudinal study concerning motor development in children with a variety of diagnoses treated with ECMO, the ones diagnosed with CDH appeared to be at the highest risk of impaired motor function (8). However, the course of motor function impairment over time in the complete spectrum of CDH-survivors is yet to be discovered (3). We hypothesized that children born with CDH are at risk for longitudinal motor function impairment. We longitudinally studied motor function, establishing total scores on motor function as well as subskills scores, together with its determinants, in a population of CDH-survivors treated either with or without ECMO.

METHODS

Patients

All children born with CDH between January 1999 and November 2007, and who had joined our prospective follow-up program in the Erasmus MC Sophia Children's Hospital, were included. This program involves assessment by an experienced pediatric physical therapist at ages 30 months, 5, 8, 12, and 17 years, including motor performance up till 12 years of age, and exercise capacity up till 17 years of age (6, 8, 13). In case of emerging motor problems, children are offered extra help; e.g., referral for physical therapy. For this study, we analyzed motor function outcomes at 5, 8, and 12 years of age. The children included in the study had all undergone at least one assessment of motor function. For organizational reasons, the assessment at 12 years of age was discontinued for non-ECMO treated patients between 2011 and 2013. Data were collected until January 2020.

Abbreviations: CDH, congenital diaphragmatic hernia; CI, confidence interval; CLD, chronic lung disease; ECMO, extracorporeal membrane oxygenation; GLM, general linear model; M-ABC, Movement Assessment Battery for Children; PICU, pediatric intensive care unit; VA, venoarterial.

Exclusion Criteria

Children were excluded if they had been diagnosed with CDH later than seven days post-partum or when the anomaly appeared to be a para-esophageal hernia or a diaphragmatic eventration. Children who could not be reliably assessed, e.g. those with a chromosomal disorder known to affect motor performance or with severe neurodevelopmental impairment, were excluded as well. As ruled by the Erasmus MC Medical Ethics Review Board, this study was exempt from the Dutch Medical Research Involving Human Subjects Act. Therefore, Medical Ethics Review Board approval was waived (MEC-2020-0551). Data acquisition took place as part of routine clinical care. Parents of included children were informed that data were evaluated.

Motor Function Assessment

Both the first and the second version of the Movement Assessment Battery for Children (M-ABC), validated for children from 3 to 16 of age, were used to assess motor performance (14, 15). The original norm scores and cutoff values are applicable to Dutch children (15). Between March 2004 and October 2012, we used the first version of the M-ABC, and from November 2012 onwards the M-ABC-2. From here on, the term M-ABC refers to both versions, whose content is similar and assumed to be comparable (16, 17). The M-ABC is divided in three age bands. Each band contains age-appropriate tests covering three domains: manual dexterity (3 items), ball skills (2 items) and balance skills (3 items).

Characteristics

We recorded the following perinatal characteristics: gender, birthweight (grams), gestational age (weeks), inborn (yes/no; yes if born in our hospital or other CDH center), prenatal diagnosis (yes/no), side of the defect, age at surgery (days), primary closure of defect (yes/no), duration of initial ventilation (days), duration of initial stay at the pediatric intensive care unit (PICU) (days), duration of initial hospital stay (days), cardiac malformations (yes/no; recorded if follow up by a pediatric cardiologist was necessary), treatment with inhaled nitric oxide (yes/no), chronic lung disease (CLD) (18), sepsis during initial hospital stay (defined as clinical suspicion confirmed with positive blood culture), ECMO-treatment (yes/no), age at start ECMO (hours), and duration of ECMO-treatment (hours), and maternal education level as classified by the International Standard Classification of Education (19).

At follow-up, the following characteristics were collected: weight-for-height z-score (20), having obtained a swimming certificate, sports participation (yes/no, yes if at least once a week, other than gymnastics at school). All characteristics were retrieved from electronic patient files.

Statistical Analysis

Mann-Whitney U tests were used to compare continuous data of participants and non-participants, who were lost to

follow up, as well as data of ECMO-treated and non-ECMO treated patients; chi-square tests were used for categorical data. All statistical tests were two-sided with a significance level of 0.05.

Raw scores from the M-ABC assessment were converted to percentile scores for clinical interpretation. For both versions of the M-ABC, a score equal to or below the fifth percentile indicates a definite motor problem. Therefore, we classified children as those who have a definite motor problem and those who have not (14, 15). The chi-square test was used to compare outcome proportions in our sample of CDH patients with normative proportions. To combine scores of both M-ABC versions, the analysis was based on the percentile scores of the M-ABC, and a probit transformation (i.e., inverse normal transformation) was performed to transform the percentile scores into z-scores (16).

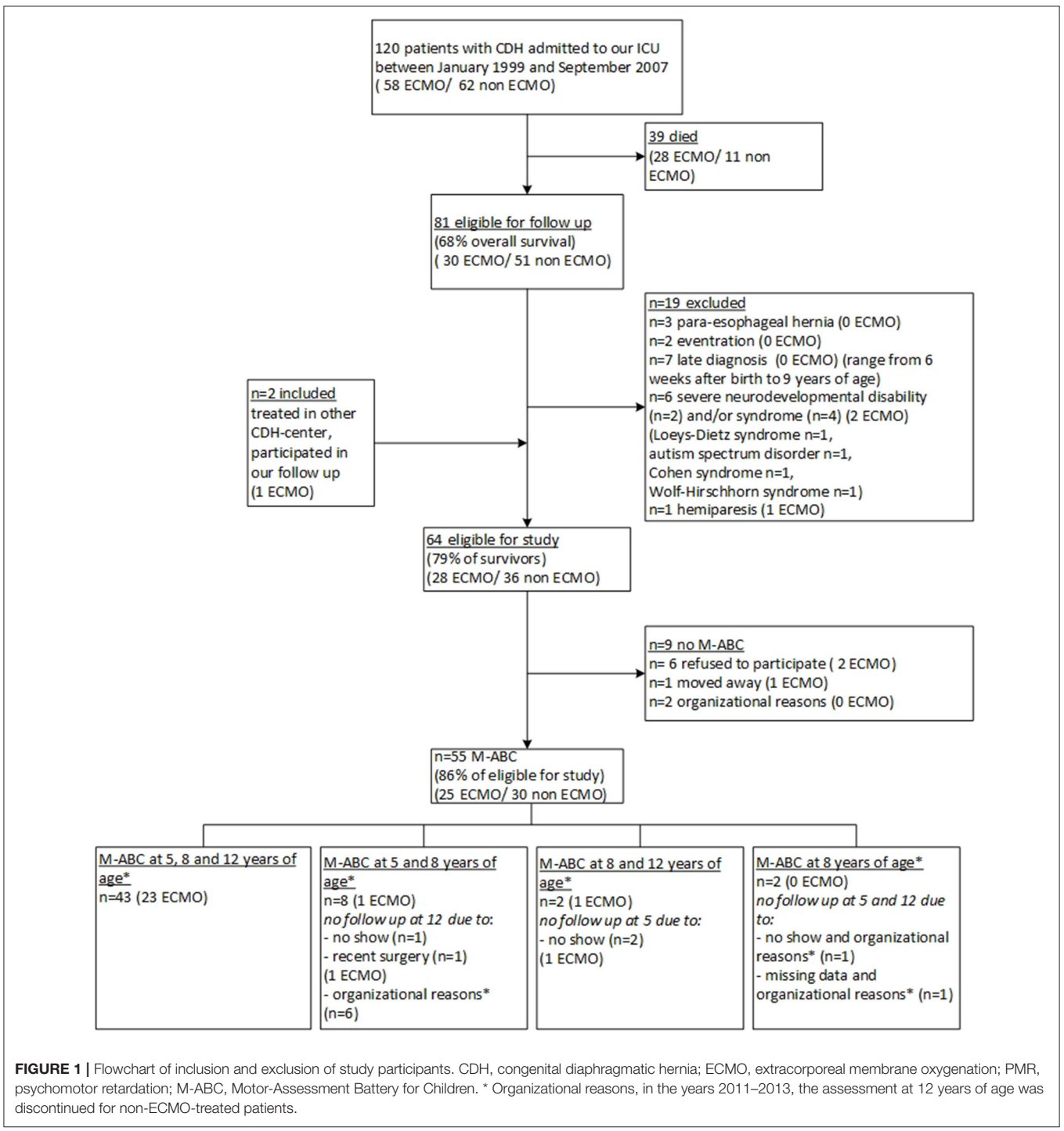

FIGURE 1 | Flowchart of inclusion and exclusion of study participants. CDH, congenital diaphragmatic hernia; ECMO, extracorporeal membrane oxygenation; PMR, psychomotor retardation; M-ABC, Motor-Assessment Battery for Children. * Organizational reasons, in the years 2011–2013, the assessment at 12 years of age was discontinued for non-ECMO-treated patients.

TABLE 1 | Background characteristics.

	Non-ECMO (n = 30) 55%		***ECMO (n = 25) 45%***		***p–value***	***Non-ECMO* n = 30 (55)**	
Boys	17	(57)	18	(72)	0.24	6	(67)
Birthweight, grams	3,000	(1,805–4,900)	3,200	(2,000–3,810)	0.12	3,000	(2,400–3,600)
Gestational age, weeks	39	(35.6–41.4)	39.3	(35.6–41.4)	0.24	39.5	(38.3–39.5)
Inborn	20	(67)	9	(36)	**0.02**	6	(67)
Prenatal diagnosis	20	(69)	10	(40)	**0.03**	6	(67)
Left sided defect	24	(80)	24	(96)	0.08	8	(89)
Age at surgery, days	4	(1–14)	11	(1–42)	**<0.001**	5	(1–20)
Primarily closed	12	(40)	4	(16)	0.05	3	(33)
Initial ventilation, days	10.5	(3–53)	28	(8–146)	**<0.001**	14	(2–64)
Initial PICU stay, days	19	(9–100)	44	(15–153)	**<0.001**	31	(8–99)
Initial hospital stay, days	32.5	(9–113)	77	(22–187)	**<0.001**	31	(15–127)
Cardiac malformations	1	(3)	3	(12)	0.22	0	(0)
Inhaled nitric oxide treatment	16	(55)	24	(96)	**<0.001**	7	(78)
Chronic lung disease	7	(23)	15	(60)	**0.01**	3[a]	(38)
Sepsis during initial hospital stay	3	(10)	9	(36)	**0.02**	3[a]	(38)
Need for ECMO	-		25	(100)		3	(33)
VA	-		25	(100)		3	(100)
Age at start ECMO, hours	-		14	(2–251)		40	(5–75)
Time on ECMO, hours	-		161	(63–369)		236	(83–237)
ISCED level mother							
Low (ISCED 0–2)	3	(10)	2	(8)	0.86	0	
Middle (ISCED 3–4)	11	(36.7)	8	(32)	0.85	0	
High (ISCED 5–8)	12	(40)	11	(44)	0.77	1	(11.1)
Not available	4	(13.3)	4	(16)		8	(88.9)

Data presented as median (range), or n (%). Non-participants: patients lost to follow up or unable to perform M-ABC. ECMO, extracorporeal membrane oxygenation; VA, venoarterial; PICU, pediatric intensive care unit; ISCED, International Standard Classification of Education (19). p-value represents comparison between non-ECMO and ECMO-treated CDH; participants and non-participants were not significantly different and p-values were not shown. [a]1 missing data. Bold values indicate significant difference between non-ECMO and ECMO-treated CDH patients.

For the longitudinal analysis of motor development over the years, we used general linear models (GLM) for repeated measurements. An advantage of GLM is that it accounts for data that are missing at random. The dependent variable in this model is the z-scores of the M-ABC. Age (coded as a categorical variable), ECMO treatment (yes/no) and their interaction effect were included as independent variables. Mean values of the M-ABC were compared between age groups, for the entire sample and stratified by ECMO group, using the estimated marginal means of this model. The determinants birthweight, CLD, initial hospital stay, primary closure of defect, sports participation and weight-for-height z-score at follow up were added to this model as independent variables, first one by one for univariate analyses and eventually all together for multivariate analysis. An unstructured covariance matrix was assumed to account for the within-patient correlations between the three age groups.

RESULTS

Between January 1999 and December 2007, 120 children born with CDH were admitted to our PICU, of whom 81 (68%) survived to date. After applying the exclusion criteria, 64 patients were eligible for this study, of whom 9 were not assessed for various reasons, resulting in 55 participants. This number includes two participants of our program who were born in another CDH-center (**Figure 1**). The clinical baseline characteristics of the participants did not differ significantly from those of the non-participants who were lost to follow up, but the frequencies of several characteristics differed significantly between ECMO-treated and non-ECMO-treated participants, such as length of stay and treatment with inhaled nitric oxide (**Table 1**). At twelve years of age, 52 children (94.5%) had obtained a swimming certificate.

Longitudinal Evaluation

The longitudinal M-ABC results of the total group had improved significantly from age 5 (estimated marginal mean z-score −0.67, 95% CI −0.96 to −0.39) towards age 8 (−0.35, 95% CI −0.65 to −0.05), resulting in a mean difference of 0.32 ($p = 0.02$), followed by a score of −0.46 at age 12 years (95% CI −0.76 to −0.17). For the non-ECMO treated patients, no significant differences in M-ABC scores were found between ages. The estimated marginal mean z-score was −0.44 at 5 years of age (95% CI −0.83 to −0.05), −0.25 at 8 years of age (95% CI −0.65 to 0.16) and −0.18 at 12 years of age (95% CI −0.59 to 0.24). For the ECMO-treated patients, the estimated marginal mean z-score increased significantly from age 5 to age 8 years, from −0.91 (95% CI −1.33 to −0.49) to −0.46 (95% CI −0.90 to −0.02), resulting in a

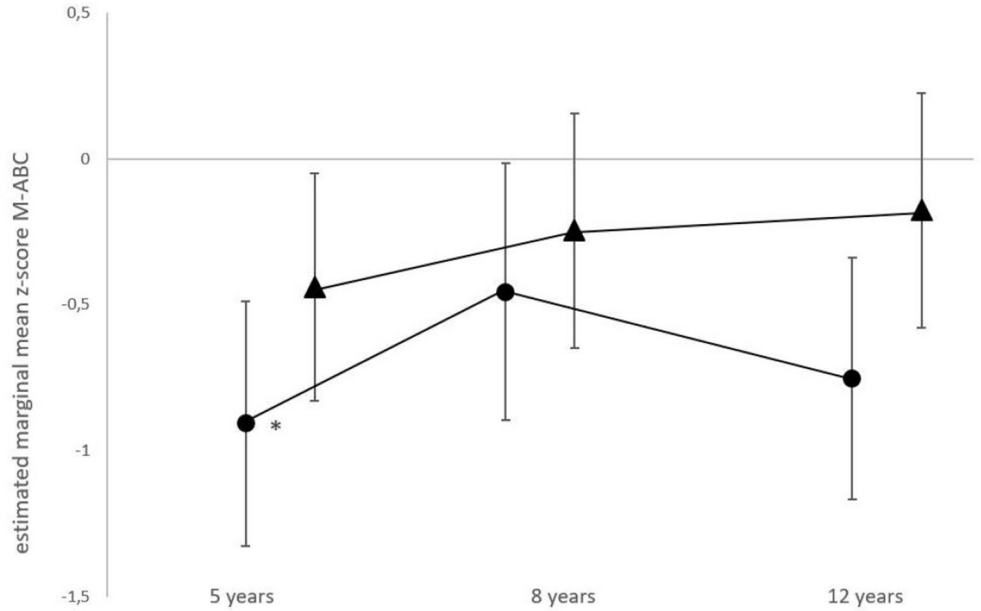

FIGURE 2 | Longitudinal motor function performance in ECMO-treated and non-ECMO-treated children with CDH. Data shown are estimated mean z-scores of the M-ABC with 95% CIs. Circles, ECMO-treated children; triangles, non-ECMO-treated children. *For the ECMO-treated patients, scores differed significantly from age 5 to age 8 years, ($p = 0.03$).

mean difference of −0.45; ($p = 0.03$) and declined at age 12 years (−0.75, 95% CI −1.17 to −0.34) (**Figure 2**).

Proportions of Motor Domain Scores Compared to Norm Population

At all three ages, the proportion of ECMO-treated children with a normal total impairment score was significantly lower than in the norm population (chi-square, all $p < 0.01$; **Table 2**).

Concerning subskills, ECMO-treated children performed significantly worse than the norm population on manual dexterity at ages 8 and 12 years ($p = 0.01$ and $p < 0.001$, respectively) and on balance skills at ages 5 and 8 years ($p < 0.001$ and $p = 0.01$, respectively). Ball skills were significantly affected in the non-ECMO treated group at age 12 years ($p = 0.003$) (**Table 2**).

Associations

Both in univariate and multivariate analysis, the length of hospital stay was negatively associated with the total z-score of M-ABC ($p = 0.001$ univariate analysis; $p = 0.004$ multivariate analysis), whereas the other variables were not significantly associated with outcome (**Table 3**).

DISCUSSION

To the best of our knowledge, this is the first study to longitudinally evaluate the course of motor function in school-aged CDH survivors. We distinguished between those who had been treated with ECMO and those who had not, since it had been shown before that ECMO-treated CDH patients were at risk for impaired motor function (8). Strikingly, at ages 8 and 12 years, the estimated mean z-scores for motor function in non-ECMO-treated participants were not significantly lower than the norm scores, whereas the scores of those treated with ECMO were. At five years of age, scores in both groups were significantly lower than the norm. In multivariate analysis, length of hospital stay was independently associated with poorer motor outcome.

Several groups have studied motor function in children with CDH. Tureczek and co-workers cross-sectionally studied outcome in 3-to-16-year-old non-ECMO-treated children born with CDH. They found that younger children, up till age five years, performed better than older children on adaptive fine and gross motor components, although this finding might have been due to the application of different tests at the various ages (21). Danzer and co-workers longitudinally evaluated motor performance within the first three years of life of children born with CDH, and reported average motor function in the majority of children (10). Church and co-workers performed a retrospective observational study in CDH survivors aged from 4 months to 7.5 years old and found overall motor function to be below average, mainly due to gross motor problems (7). Overall, despite the variability in type of assessment and age of testing, all studies in CDH survivors are consistent in the occurrence of gross motor problems.

Earlier studies in CDH as well as ECMO-survivors revealed need for methadone, CLD and lower observed-to-expected total fetal lung volume as determinants for motor function problems (6–8). In our study, length of hospital was negatively associated with motor function, whereas CLD was not. This association has previously been found in survivors of CDH at toddler age

TABLE 2 | Characteristics and results at follow up.

	5 years $n = 51$		8 years $n = 55$		12 years $n = 45$	
	Non-ECMO $n = 27$ (53)	**ECMO $n = 24$ (47)**	**Non-ECMO $n = 30$ (55)**	**ECMO $n = 25$ (45)**	**Non-ECMO $n = 21$ (47)**	**ECMO $n = 24$ (53)**
Boys, (n)	15 (56)	17 (71)	17 (56)	18 (72)	12 (57)	17 (71)
Weight-for-height, z-score	−0.73 (−2.96 to 1.55)	−1.60 (−3.69 to 0.01)	−0.50 (−2.53 to 1.20)	−1.44 (−3.86 to 1.23)	−0.30 (−2.43 to 1.8)	−0.70 (−3.37 to 2.17)
Sports participation, (n)	16 (59)	14 (58)	26 (87)	17 (68)	16 (76)	14 (58)
M-ABC total impairment score						
normal – borderline, > p5 (n)	24 (89)	18 (75)*	27 (90)	19 (76)*	20 (95)	18 (75)*
definite motor problem, p≤5 (n)	3 (11)	6 (25)	3 (10)	6 (24)	1 (5)	6 (25)
M-ABC manual dexterity						
normal – borderline, > p5 (n)	26 (96)	21 (87.5)	28 (93)	21 (84)^	19 (90.5)	18 (75)*
definite motor problem, p≤5 (n)	1 (4)	3 (12.5)	2 (7)	4 (16)	2 (9.5)	6 (25)
M-ABC ball skills						
normal-borderline, > p5 (n)	25 (93)	21 (87.5)	28 (93)	22 (88)	17 (81)*	22 (92)
definite motor problem, $p \leq 5$ (n)	2 (7)	3 (12.5)	2 (7)	3 (12)	4 (19)	2 (8)
M-ABC balance skills						
normal – borderline, > p5 (n)	24 (89)	18 (75)*	28 (93)	21 (84)^	19 (90.5)	22 (92)
definite motor problem, $p \leq 5$ (n)	3 (11)	6 (25)	2 (7)	4 (16)	2 (9.5)	2 (8)

*P, percentile. Data shown n (%) or median (range). ECMO, extracorporeal membrane oxygenation. M-ABC, Movement assessment battery for children. *p < 0.01 Chi-square test in comparison with norm values. ^p < 0.05 Chi-square test in comparison with norm values.*

TABLE 3 | Possible determinants of motor performance in the total group of children with CDH.

Independent variables	**z-scores of the M-ABC**					
	Univariate analysis			**Multivariate analysis**		
	β	**95% CI**	***p*-value**	**β**	**95% CI**	***p*-value**
Birthweight (kg)	0.36	−0.09 to 0.81	0.11	0.31	−0.13 to 0.74	0.16
CLD (yes/no)	−0.45	−0.98 to 0.09	0.10	0.17	−0.51 to 0.84	0.62
Initial hospital stay (days)[a]	**−0.01**	−0.02 to −0.004	**0.001**	**−0.01**	−0.02 to −0.005	**0.004**
Primary closure of defect (yes/no)	−0.09	−0.66 to 0.48	0.76	−0.23	−0.77 to 0.31	0.40
weight–for–height z-score at follow-up	−0.05	−0.21 to 0.12	0.58	−0.13	−0.30 to 0.03	0.10
Sports participation (yes/no)	0.13	−0.17 to 0.42	0.40	0.12	−0.17 to 0.41	0.41

CLD, chronic lung disease as defined by Jobe and Bancalari (18). Results are based on a general linear model, which has been adjusted for ECMO-treatment and age, as well as for interaction between those two variables. [a]Significant association. Bold values indicate significant association.

(9, 10). The results of our multivariable analysis suggest that the z-score of M-ABC decreases with 0.01 for every week of initial hospitalization, but our model does not allow to determine a critical threshold of length of hospital stay to predict need of follow-up of motor function. Nevertheless, we propose that risk-stratification should not rely solely on ECMO-treatment, but include length of hospital stay as well. Longer length of stay itself might not be contributive to impaired motor function; it rather reflects severity of disease with underlying problems, such as pulmonary hypertension and failure to thrive.

The five-year-olds in our study appeared to be at risk for poor motor functioning, but motor performance improved thereafter – especially in CDH-survivors who had not received ECMO-treatment. Our data do not allow to conclude what actually has contributed to the improvement, although enrolment in our longitudinal follow-up program, with more timely referral to pediatric physical therapists and active stimulation to sports participation, is likely to have contributed (6, 8, 12, 22).

A few issues relating to this study need to be addressed. First, all but one of the included children underwent laparotomy, which in the study period (1999–2007) was standard of care for surgical correction of CDH. Nowadays, more infants undergo minimal access surgery (23). The question whether gross motor function might be affected by impaired truncal muscle strength after laparotomy has not yet been answered, but deserves further investigation. Second, children with CDH born today are treated with a standardized perinatal protocol introduced

in 2008 (24). This protocol resulted in a decline in both mortality and need for ECMO (25, 26). Moreover, CDH is more and more predicted prenatally through standardized ultrasound examination at 20 weeks gestational age. The large majority of the participants in our study were born before the introduction of the protocol and the standardized ultrasound, so that the studied children overall are not fully representative of patients born today.

Although we have previously reported that CDH survivors at school age are at risk for pulmonary morbidity (27), we did not include data concerning lung function. In a randomized controlled trial involving CDH patients with airflow obstruction, we concluded that both exercise tolerance and motor function improved irrespective of intervention, and that parental awareness of reduced exercise capacity rather than specific interventions may have contributed to the improvement (28). This might indicate that lifestyle factors, rather than decreased lung function, contribute to impaired motor function.

Regarding awareness, both the child and its parents tend overestimate the child's motor competence as compared to the results of the M-ABC (29, 30). After having seen their child in a state of critical illness, the urge for well-developed motor function might seem futile to parents (29). Additionally, some parents might consider their child too vulnerable to actively participate in sports. Yet, this underlines the importance of comprehensive counseling of parents.

Moreover, treatment-related causes of motor function impairment might play a role, too. Abnormal cerebral ultrasound findings are not uncommon in this group, especially in ECMO-treated patients, although those findings are not linked to motor development yet (8, 31). Future studies concerning outcome should include the results of close neuromonitoring, as is currently being done in the NEMO-trial (NTR7160), which aims to gain more insight in the physiology of the brain of CDH-patients perioperatively. Also, neuroimaging during the period of critical illness as determinant for future motor development could be of use, to allow detailed risk stratification and prognostication.

Several limitations of our study must be taken into account. First, over the study period, two consecutive versions of the M-ABC were applied. Nevertheless, converting the scores to z-scores allowed comparison between test results (16). Second, this is a single-center study, which limits the transferability of the results. Third, we did not include a score for critical illness, which limited the identification of predictive determinants for impaired motor function. In a previous study by our group, the vaso-active inotropic score (VIS) was found to be predictive of neurocognitive outcome in ECMO-treated patients, and we therefore recommend to take this score into account in further studies concerning development (32). Until 2005 cumulative drug doses were lacking as digital records were unavailable. We were therefore unable to properly retrieve data and calculate VIS for our cohort. Other factors that might have been of interest but were not taken into account in our analyses were social and environmental characteristics, such as time outdoors, availability of toys and space to play, which have been reported to influence motor development (33, 34). We were unable to use those since these data were not collected in the past. However, taking into account the sociodemographic background of the participants (ISCED level mother, **Table 1**) and the policy of the Dutch government to stimulate sports and swimming classes for low income families, we think that this may not have had an important role. Moreover, we did not investigate cognitive competence and school achievement in this study. Yet, our group has previously studied neurocognitive outcome in school-aged CDH-survivors with and without ECMO, and found problems on several neurocognitive domains. Therefore, persisting problems on several neurodevelopmental domains, such as motor and cognitive function, warrant further evaluation regarding the growing into deficit theory (32).

The strengths of our study can be found in the relatively large cohort with a follow up of 12 years, and the fact that 86% of the eligible candidates actually participated in our follow-up. We found no evidence for selection bias, since the baseline characteristics between the non-participants and participants did not significantly differ.

CONCLUSION

In conclusion, children born with CDH are at risk for motor function impairment at the age of five, and impairment may persist up till 12 years of age in those who were treated with ECMO. Length of hospital stay appeared to be an independent risk factor for impaired motor function. Early recognition of motor problems and timely referral to pediatric physical therapists could help prevent worsening. A clinical implication of our findings is that motor function should be monitored up to five years of age in all CDH-survivors. The decision to extend follow up until adolescence should take both need for ECMO-treatment and prolonged hospital stay into account.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are currently not available. Requests to access the datasets should be directed to h.ijsselstijn@erasmusmc.nl.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical Ethical Review Committee Erasmus MC Rotterdam. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

SdM contributed to the conception and design, acquisition of data, analysis and interpretation of data, and writing of the

first draft. MvdC-vZ and HI contributed to the conception and design, acquisition of data, analysis and interpretation of data, writing of the first draft, and critically revising the manuscript. TZ-vdA contributed to acquisition of data and critically revising the manuscript. SC-dO and NvH contributed to interpretation of data and critically revising the manuscript. RW and SG contributed to the conception and design, and critically revising the manuscript. JvR contributed to statistical analysis, interpretation of the data and critically revising the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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REFERENCES

1. McGivern MR, Best KE, Rankin J, Wellesley D, Greenlees R, Addor MC, et al. Epidemiology of congenital diaphragmatic hernia in Europe: a register-based study. *Arch Dis Child Fetal Neonatal Ed.* (2015) 100:F137–44. doi: 10.1136/archdischild-2014-306174
2. Snoek KG, Greenough A, van Rosmalen J, Capolupo I, Schaible T, Ali K, et al. Congenital diaphragmatic hernia: 10-year evaluation of survival, extracorporeal membrane oxygenation, and foetoscopic endotracheal occlusion in four high-volume centres. *Neonatology.* (2018) 113:63–8. doi: 10.1159/000480451
3. Montalva L, Raffler G, Riccio A, Lauriti G, Zani A. Neurodevelopmental impairment in children with congenital diaphragmatic hernia: not an uncommon complication for survivors. *J Pediatr Surg.* (2020) 55:625–34. doi: 10.1016/j.jpedsurg.2019.05.021
4. Spoel M. van der Cammen-van Zijp MH, Hop WC, Tibboel D, de Jongste JC, Ijsselstijn H. Lung function in young adults with congenital diaphragmatic hernia a longitudinal evaluation. *Pediatr Pulmonol.* (2013) 48:130–7. doi: 10.1002/ppul.22557
5. van der Cammen-van Zijp MH, Spoel M, Laas R, Hop WC, de Jongste JC, Tibboel D, et al. Exercise capacity, daily activity, and severity of fatigue in term born young adults after neonatal respiratory failure. *Scand J Med Sci Sports.* (2014) 24:144–51. doi: 10.1111/j.1600-0838.2012.01491.x
6. Madderom MJ, Toussaint L. van der Cammen-van Zijp MH, Gischler SJ, Wijnen RM, Tibboel D, et al. Congenital diaphragmatic hernia with(out) ECMO: impaired development at 8 years. *Arch Dis Child Fetal Neonatal Ed.* (2013) 98:F316–22. doi: 10.1136/archdischild-2012-303020
7. Church JT, Mon R, Wright T, Coughlin MA, Ladino-Torres M, Tapley C, et al. Neurodevelopmental outcomes in CDH survivors: a single institution's experience. *J Pediatr Surg.* (2018) 53:1087–91. doi: 10.1016/j.jpedsurg.2018.02.064
8. van der Cammen-van Zijp MH, Janssen AJ, Raets MM, van Rosmalen J, Govaert P, Steiner K, et al. Motor performance after neonatal extracorporeal membrane oxygenation: a longitudinal evaluation. *Pediatrics.* (2014) 134:e427–35. doi: 10.1542/peds.2013-3351
9. Snoek KG, Capolupo I, Braguglia A, Aite L, van Rosmalen J, Valfre L, et al. Neurodevelopmental outcome in high-risk congenital diaphragmatic hernia patients: an appeal for international standardization. *Neonatology.* (2016) 109:14–21. doi: 10.1159/000438978
10. Danzer E, Gerdes M, D'Agostino JA, Hoffman C, Bernbaum J, Bebbington MW, et al. Longitudinal neurodevelopmental and neuromotor outcome in congenital diaphragmatic hernia patients in the first 3 years of life. *J Perinatol.* (2013) 33:893–8. doi: 10.1038/jp.2013.47
11. Friedman S, Chen C, Chapman JS, Jeruss S, Terrin N, Tighiouart H, et al. Neurodevelopmental outcomes of congenital diaphragmatic hernia survivors followed in a multidisciplinary clinic at ages 1 and 3. *J Pediatr Surg.* (2008) 43:1035–43. doi: 10.1016/j.jpedsurg.2008.02.029
12. van der Cammen-van Zijp MH, Gischler SJ, Mazer P, van Dijk M, Tibboel D, Ijsselstijn H. Motor-function and exercise capacity in children with major anatomical congenital anomalies: an evaluation at 5 years of age. *Early Hum Dev.* (2010) 86:523–8. doi: 10.1016/j.earlhumdev.2010.06.014
13. Toussaint-Duyster LCC. van der Cammen-van Zijp MHM, de Jongste JC, Tibboel D, Wijnen RMH, Gischler SJ, et al. Congenital diaphragmatic hernia and exercise capacity, a longitudinal evaluation. *Pediatr Pulmonol.* (2019) 54:628–36. doi: 10.1002/ppul.24264
14. Henderson FS. *The Movement Assessment Battery for Children: Manual.* The psychological corporation (1992).
15. Smits-Engelsman B. *Movement abc-2-nl.* Dutch manual Amsterdam: Pearson. (2010).
16. Harmsen WJ, Aarsen FJ, van der Cammen-van Zijp MHM, van Rosmalen JM, Wijnen RMH, Tibboel D, et al. Developmental problems in patients with oesophageal atresia: a longitudinal follow-up study. *Arch Dis Child Fetal Neonatal Ed.* (2017) 102:F214–F9. doi: 10.1136/archdischild-2015-309976
17. Smits-Engelsman B, Hill EL. The relationship between motor coordination and intelligence across the IQ range. *Pediatrics.* (2012) 130:e950–6. doi: 10.1542/peds.2011-3712
18. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *Am J Respir Crit Care Med.* (2001) 163:1723–9. doi: 10.1164/ajrccm.163.7.2011060
19. Statistics UIo. International Standard CLassification of Education (ISCED) (2011) Available online at: http://uis.unesco.org/en/topic/international-standard-classification-education-isced.
20. Talma H. *Groeidiagrammen 2010: handleiding bij het meten en wegen van kinderen en het invullen van groeidiagrammen.* Leiden, the Netherlands TNO. (2010).
21. Tureczek I, Caflisch J, Moehrlen U, Natalucci G, Bernet V, Latal B. Long-term motor and cognitive outcome in children with congenital diaphragmatic hernia. *Acta Paediatr.* (2012) 101:507–12. doi: 10.1111/j.1651-2227.2011.02567.x
22. Gischler SJ, Mazer P, Duivenvoorden HJ, van Dijk M, Bax NM, Hazebroek FW, et al. Interdisciplinary structural follow-up of surgical newborns: a prospective evaluation. *J Pediatr Surg.* (2009) 44:1382–9. doi: 10.1016/j.jpedsurg.2008.12.034
23. Snoek KG, Reiss IK, Greenough A, Capolupo I, Urlesberger B, Wessel L, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH EURO consortium consensus - 2015 update. *Neonatology.* (2016) 110:66–74. doi: 10.1159/000444210
24. Reiss I, Schaible T, van den Hout L, Capolupo I, Allegaert K, van Heijst A, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH EURO consortium consensus. *Neonatology.* (2010) 98:354–64. doi: 10.1159/000320622
25. Paden ML, Conrad SA, Rycus PT, Thiagarajan RR, Registry E. Extracorporeal life support organization registry report 2012. *ASAIO J.* (2013) 59:202–10. doi: 10.1097/MAT.0b013e3182904a52
26. van den Hout L, Schaible T, Cohen-Overbeek TE, Hop W, Siemer J, van de Ven K, et al. Actual outcome in infants with congenital diaphragmatic hernia: the role of a standardized postnatal treatment protocol. *Fetal Diagn Ther.* (2011) 29:55–63. doi: 10.1159/000322694
27. Toussaint-Duyster LCC, van der Cammen-van Zijp MHM, Spoel M, Tiddens H, Tibboel D, Wijnen RMH, et al. Lung function in school-aged congenital diaphragmatic hernia patients a longitudinal evaluation. *Pediatr Pulmonol.* (2019) 54:1257–66. doi: 10.1002/ppul.24375
28. Toussaint-Duyster LCC, van der Cammen-van Zijp MHM, Takken T, Harmsen WJ, Tibboel D, van Heijst AFJ, et al. Improvement of exercise capacity following neonatal respiratory failure: a randomized controlled trial. *Scand J Med Sci Sports.* (2020) 30:662–71. doi: 10.1111/sms.13604
29. Toussaint-Duyster LCC, van der Cammen-van Zijp MHM, Tibboel D, Gischler S, van Rosmalen J, IJsselstijn H. A parent-reported standardised checklist is not sensitive to screen for motor problems at school age following neonatal critical illness. *Acta Paediatr.* (2020) 109:1801–6. doi: 10.1111/apa.15192

30. Toussaint LC, van der Cammen-van Zijp MH, Janssen AJ, Tibboel D, van Heijst AF, IJsselstijn H. Perceived motor competence differs from actual performance in 8-year-old neonatal ECMO survivors. *Pediatrics.* (2016) 137:e20152724. doi: 10.1542/peds.2015-2724
31. Raets MM, Dudink J, Ijsselstijn H, van Heijst AF, Lequin MH, Houmes RJ, et al. Brain injury associated with neonatal extracorporeal membrane oxygenation in the Netherlands: a nationwide evaluation spanning two decades. *Pediatr Crit Care Med.* (2013) 14:884–92. doi: 10.1097/PCC.0b013e3182a555ac
32. Leeuwen L, Schiller RM, Rietman AB, van Rosmalen J, Wildschut ED, Houmes RJM, et al. Risk factors of impaired neuropsychologic outcome in school-aged survivors of neonatal critical illness. *Crit Care Med.* (2018) 46:401–10. doi: 10.1097/CCM.0000000000002869
33. Zeng N, Johnson SL, Boles RE, Bellows LL. Social-ecological correlates of fundamental movement skills in young children. *J Sport Health Sci.* (2019) 8:122–9. doi: 10.1016/j.jshs.2019.01.001
34. Barnett LM, Hnatiuk JA, Salmon J, Hesketh KD. Modifiable factors which predict children's gross motor competence: a prospective cohort study. *Int J Behav Nutr Phys Act.* (2019) 16:129. doi: 10.1186/s12966-019-0888-0

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CASE REPORT
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Case Report and Review of the Literature: Congenital Diaphragmatic Hernia and Craniosynostosis, a Coincidence or Common Cause?

Linda Gaillard[1], Anne Goverde[2], Quincy C. C. van den Bosch[2], Fernanda S. Jehee[2], Erwin Brosens[2], Danielle Veenma[2], Frank Magielsen[2], Annelies de Klein[2], Irene M. J. Mathijssen[1†] and Marieke F. van Dooren[2†]*

[1] Department of Plastic, Reconstructive and Hand Surgery, Erasmus Medical Center—Sophia Children's Hospital, University Medical Center Rotterdam, Rotterdam, Netherlands, [2] Department of Clinical Genetics, Erasmus Medical Center—Sophia Children's Hospital, University Medical Center Rotterdam, Rotterdam, Netherlands

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***Correspondence:**
Linda Gaillard
l.gaillard@erasmusmc.nl

† These authors share last authorship

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Congenital diaphragmatic hernia (CDH) is a life-threatening birth defect that presents as either an isolated diaphragm defect or as part of a complex disorder with a wide array of anomalies (complex CDH). Some patients with complex CDH display distinct craniofacial anomalies such as craniofrontonasal dysplasia or craniosynostosis, defined by the premature closure of cranial sutures. Using clinical whole exome sequencing (WES), we found a *BCL11B* missense variant in a patient with a left-sided congenital diaphragmatic hernia as well as sagittal suture craniosynostosis. We applied targeted sequencing of *BCL11B* in patients with craniosynostosis or with a combination of craniosynostosis and CDH. This resulted in three additional *BCL11B* missense mutations in patients with craniosynostosis. The phenotype of the patient with both CDH as well as craniosynostosis was similar to the phenotype of previously reported patients with *BCL11B* missense mutations. Although these findings imply that both craniosynostosis as well as CDH may be associated with *BCL11B* mutations, further studies are required to establish whether *BCL11B* variants are causative mutations for both conditions or if our finding was coincidental.

Keywords: case report, craniosynostosis, congenital diaphragmatic hernia (CDH), BCL11B, craniosynostosis syndromes

INTRODUCTION

The genetic etiology of congenital diaphragmatic hernia (CDH) is complex. Structural variants, small and large insertions or deletions and single-nucleotide variants (SNVs) in over a 100 genes have been associated with CDH (1). Only a few of these genes are mutated recurrently in multiple individuals and even then the phenotype can differ largely due to incomplete penetrance. CDH has a prevalence of 2.3–2.7 per 10.000 live births (2–4) and can present as isolated CDH or in association with additional congenital anomalies (non-isolated CDH or complex CDH), as seen in ∼40 (5) to 49% (6) of the cases. Complex CDH may present as part of a recognizable genetic syndrome, chromosome abnormality, or a collection of major congenital malformations. One of the less common malformations in patients with CDH are craniofacial anomalies and in particular craniosynostosis.

Although less common in syndromes associated with complex CDH, several studies have described syndromes such as Apert's syndrome and Craniofrontonasal syndrome, that include both CDH as well as craniosynostosis as cardinal or relatively common features (7–22). Craniosynostosis, a developmental disorder defined by the premature fusion of one or more sutures, has a prevalence of 7.2 per 10,000 live births (23). It can be divided into non-syndromic and syndromic craniosynostosis, with syndromic craniosynostosis being characterized by additional congenital anomalies, such as limb anomalies and neurodevelopmental delays (24, 25). As part of our standard clinical care, all patients diagnosed with craniosynostosis are offered targeted genetic analysis (26). In a female patient with both CDH as well as craniosynostosis, we observed a genetic variant of the B cell leukemia 11b gene (*BCL11B;* OMIM 606558) resulting in an amino acid change at position 667 [p.(Gly667Glu)].

Searching the literature for BCL11B mutations we found several case reports. First, a case report described a male patient with a heterozygous *de novo* missense *BCL11B* mutation (p.Asn441Lys), who presented with severe combined immunodeficiency as well as neurological, dermal and facial dysmorphisms including hypertelorism, short palpebral fissures and micrognathia (27). Second, a study reported on 13 patients with heterozygous germline mutations in *BCL11B* (28). Most of these patients presented with neurodevelopmental disorders and immunodeficiency with reduced type 2 innate lymphoid cell and were carriers of loss of function mutations in BCL11B. However, in the most severely affected patient (patient EII-I) a missense BCL11B mutation p.(Asn807Lys) was found. This patient had a similar phenotype as compared to the first patient described by Punwani et al. (27) including a myopathic facial appearance, hypertelorism and small palpebral fissures. Neither study reported on the presence of craniosynostosis or CDH although craniofacial anomalies were described for patient EII-I and are apparent for the patient described by Punwani et al. (27). However, we previously discovered a *de novo BCL11B* missense mutation in exon 1 which encodes for [p.(Arg3Ser)] in a male patient with unilateral coronal suture craniosynostosis (29). In addition, a *de novo BCL11B* missense mutation in exon 4 [p.(Arg350Cys)] was reported in a patient who presented with CDH, an abnormal optic nerve, increased intraocular pressure and scoliosis (30). These findings suggest that both craniosynostosis as well as CDH may be associated with *BCL11B missense* mutations.

For this report, we selected patients who had undergone genetic testing with craniosynostosis or combined CDH and craniosynostosis from the Erasmus MC- Sophia Children's hospital. This search resulted in three additional *BCL11B* missense variants. In this report we present the clinical reports of these four new patients with *BCL11B* variants and a brief review of disorders that are characterized by both CDH as well as craniosynostosis. Informed consent to publish case descriptions and images was obtained from patients, and/or their parents if applicable. All genetic information is provided according to the HGNC guidelines (31).

CLINICAL REPORTS

Patient A

Patient A [NM_138576.3(BCL11B):c.2000G>A, p.(Gly667Glu)], a 30 year old female, is the third child of healthy non-consanguineous parents, born at 41 weeks of gestation with a birth weight of ∼2,200 g, following an uncomplicated pregnancy. Shortly after birth, the patient developed severe respiratory insufficiency, resulting in apnea and asystole. After resuscitation, the patient was transferred to our tertiary care center. She presented with left-sided CDH, resulting in respiratory insufficiency and pulmonary hypertension, for which she underwent surgery the second day after birth. She was weaned off respiratory support after 2 weeks. She showed several dysmorphic features including, slight ocular proptosis, hypertelorism, down slanting palpebral fissures, ptosis, arched eyebrows, and syndactyly of the second and third toes of both feet (**Figures 1A–C**). In addition, she suffered from feeding difficulties and gastroesophageal reflux due to pyloric stenosis, which was surgically corrected at the age of 2 months. She remained hypotonic and developed psychomotor delays during the first year of life. At the age of 11 months she was diagnosed with progressive sagittal suture craniosynostosis, for which she underwent surgery at the ages of 14 and 16 months. During surgery part of the calvarium looked abnormal and was sent for pathological investigation, revealing a capillary and cavernous hemangioma. She developed divergent strabismus, latent nystagmus, hypermetropia and astigmatism, and suffered from recurrent episodes of pneumonia, sinusitis and rhinitis. She showed no signs of severe cognitive impairment.

Patient B

Patient B [NM_138576.3(BCL11B): c.1744G>A, p.(Gly582Ser)], a 16 year old male, is the second child of unaffected non-consanguineous parents. He was born at 40 weeks of gestation, with a birth weight of 3,710 g. He presented with craniosynostosis of the sagittal suture and both lambdoid sutures, for which he underwent surgery at the age of 6 months. In addition, he suffered from an incomplete left-sided unilateral cleft lip including the alveolar arch, which was surgically corrected in three stages (at the ages of 6 months, 6, and 11 years). He showed several dysmorphic features, including frontal bossing, down-slanting palpebral fissures, hypotelorism, mild webbing of the neck, and hyperpigmentation on the left shoulder (**Figures 1D–I**). Magnetic Resonance Imaging (MRI) of the brain at the age of 8 years showed a Chiari I malformation. Ophthalmological assessment revealed astigmatism and myopia. He developed a proportionate short stature of −2.5 to 2 SD with delayed bone age, for which puberty was postponed using leuprorelin and letrozole. He had no neurodevelopmental delays. Motor and speech development were within normal range although he required speech therapy and physical therapy. He developed Kawasaki disease at the age of 7 years, which responded well to intravenous immunoglobulins. At the age of 16 years he underwent a second cranial vault surgery including biparietal expansion and occipital decompression. He did not suffer from frequent infections.

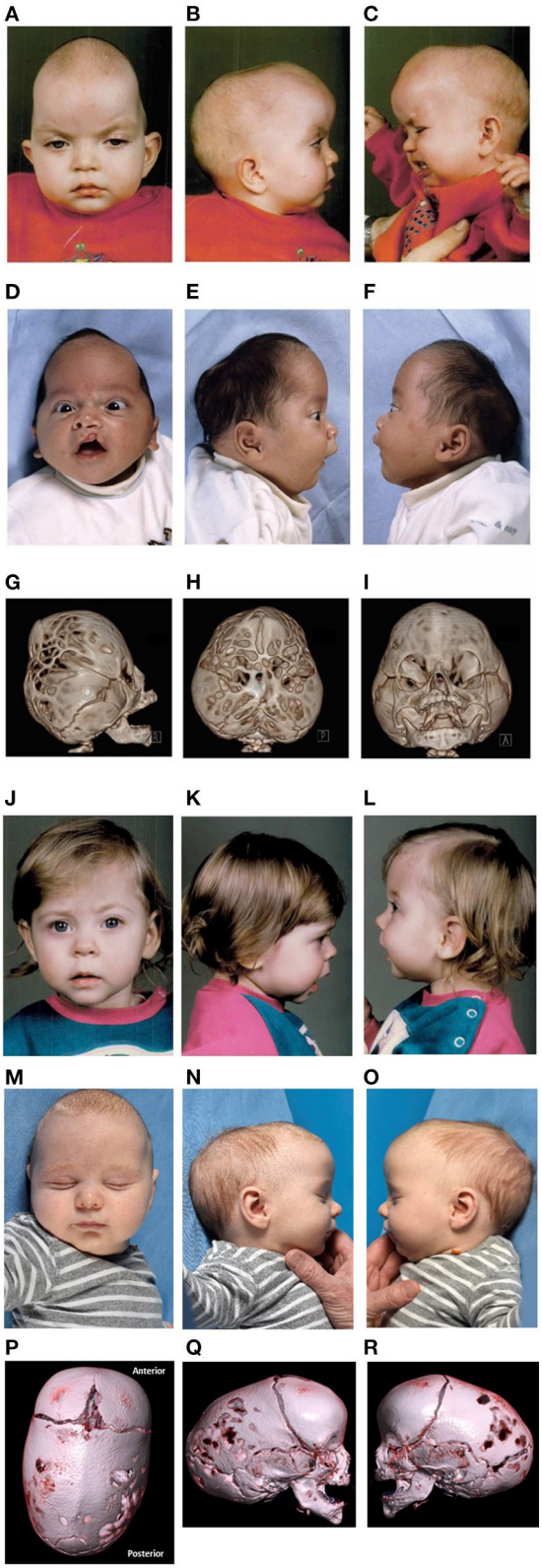

FIGURE 1 | Clinical features. Photos published with consent. **(A–C)** Pre-operative features of patient A at the age of 14 months. Historic

(Continued)

FIGURE 1 | pre-operative radiological imaging could not be retrieved of the CDH and craniosynostosis. **(D–I)** Pre-operative features and 3DCT-scan imaging of patient B at the age of 1 and 6 months, respectively. **(J–L)** Pre-operative features of patient C at the age of 12 months. Historic pre-operative radiological imaging of the craniosynostosis could not be retrieved. **(M–R)** Pre-operative features and 3DCT-scan imaging of patient D at the age of 2 months.

Patient C

Patient C [NM_138576.3(BCL11B):c.2018C>G, p.(Pro673Arg)], a 30 year old female, is the child of non-consanguineous phenotypically normal parents. A sibling and paternal half-sibling are both healthy. She presented with left-sided unicoronal craniosynostosis, for which she underwent surgery at the age of 14 months. She developed no neurodevelopmental delays and did not suffer from recurrent infections to our knowledge, although the cranial vault operation was postponed twice due to upper airway infections. She required glasses from the age of 8 years onwards. She did not display any evident dysmorphic features with the exception of mild vertical orbital dystopia (**Figures 1J–L**).

Patient D

Patient D [NM_138576.3(BCL11B):c.1265C>T, p.(Pro422Leu)], a 2 year old male, is the second child of unaffected non-consanguineous parents. In addition, the patient has two unaffected paternal half-siblings. He was born with a birth weight of 4,230 g at 41.3 weeks of gestation. He was born with the umbilical cord wrapped around his neck but recovered well after stimulation and did not require oxygen support. The pregnancy was otherwise uncomplicated. He presented with craniosynostosis of the sagittal suture at the outpatient clinic at the age of 3 months for which he underwent spring-assisted surgery at the age of 6 months. He had no neurodevelopmental delays and did not suffer from recurrent infections. Dysmorphic features were mild and included thick alae nasi and mild retrognathia with an overbite (**Figures 1M–R**).

OVERVIEW OF PATIENTS WITH BCL11B MISSENSE MUTATIONS AND VARIANTS

An overview of all patients described above, including genetic and phenotypic information of each patient can be found in **Table 1**. In addition, it shows the previously described patients with *BCL11B missense* mutations**.** Phenotypical features vary heavily among patients. The patient, reported by Goos et al. (29), displayed a similar phenotype to our Patient C, with both patients presenting with coronal suture synostosis without neurodevelopmental delays or other severe associated features. Patient A and patient B, are more similar to patients reported by Punwani et al. (27) and Lessel et al. (28). They have a more severe phenotype, presenting with a combination of multi-sutural craniosynostosis, CDH and cleft. In an additional study, one patient with a *de novo BCL11B* missense mutation [p.(Arg350Cys)] was reported to have presented with complex

TABLE 1 | Case descriptions of patients with BCL11B missense variants: genetic and phenotypical features.

	Patient A	**Patient B**	**Patient C**	**Patient D**	**Goos et al. (29)**	**Lessel et al. (28) (E:II-1)**	**Punwani et al. (27)**	**Longoni et al. (30) (T45)**
Nucleotide change*	c.2000G>A	c.1744G>A	c.2018C>G	c.1265C>T	c.7C>A	c.2421C>G	c.1323T>G	c.C1048T
Protein change	p.(Gly667Glu)	p.(Gly582Ser)	p.(Pro673Arg)	p.(Pro422Leu)	p.(Arg3Ser)	p.(Asn807Lys)	p.(Asn441Lys)	p.(Arg350Cys)
Exon	4	4	4	4	1	4	4	4
CADD score‡	18.89	3.944	22.7	22.6	24.5	25.5	24.8	31
Type of variant	Missense**	Missense**	Missense	Missense	Missense	Missense	Missense	Missense
Mode of inheritance	Maternal	Paternal	Maternal	Maternal	*De novo*	*De novo*	*De novo*	*De novo*
Phenotype								
Gastro-Intestinal								
CDH	Left-sided	–	–	–	–	–	–	+, complex
Feeding difficulties	+	–	–	–	–	+	NR	NR
Pyloric stenosis	+	–	–	–	–	+	NR	NR
Gastroesophageal reflux	+	–	–	–	–	+	NR	NR
Skeletal								
Craniosynostosis	Sagittal	Sagittal, lambdoid (bilateral)	Coronal, left	Sagittal	Coronal, right	–	–	NR
Cognition, behavior, and motor development								
Intellectual disability	–	–	–	–	–	+	+	NR
Speech impairment	+	Speech therapy required	–	–	–	+	+	NR
Delay in motor development	+	Physical therapy required	–	–	–	+	+	NR
Dysmorphic features								
Myopathic facial appearance	+	–	–	–	–	+	NR	NR
Eyebrow anomalies	Arched	–	–	–	Narrow	–	NR	NR
Small palpebral fissures	–	–	–	–	–	+	+	NR
Hypertelorism	+	–	–	–	–	+	+	NR
Hypotelorism	–	+	–	–	–	–	–	NR
Prominent nose	–	Asymmetric nose	–	Thick alae nasi	Short nose	+, upturned	NR	NR
Long philtrum	+	–	+	–	+	–	NR	NR
Lip anomalies	Full lower lip	Incomplete unilateral cleft lip and alveolar arch	Full lower lip	–	–	Thin upper lip and vermilion; down-turned corners, small mouth	NR	NR

(Continued)

TABLE 1 | Continued

	Patient A	Patient B	Patient C	Patient D	Goos et al. (29)	Lessel et al. (28) (E:II-1)	Punwani et al. (27)	Longoni et al. (30) (T45)
Ptosis	+	+	–	–	–	NR	NR	NR
Downslant of the eyes	+	+	–, upslant	–	–	–, upslant	NR	NR
Eversion of the lower eyelids	+	+	–	–	–	NR	NR	NR
Vertical orbital dystopia	–	–	+	–	+	NR	NR	NR
Retrognathia	–	+, mild	+, mild	+, mild	–	Micrognathia	Micrognathia	NR
Dysmorphic features								
Dermatological anomalies	Eczematous skin	Hirsutism	–	–	–	Severe congenital erosive dermatitis	Erythematous psoriaform dermatitis hirsutism	NR
Ear anomalies	Low-set ears	Low-set ears, fleshy upturned earlobes	–	–	–	Posteriorly rotated ears	Ear tag	NR
Other	Deep-set eyes	Deep-set eyes	Mild asymmetry of the eyes; deep-set eyes; periorbital fullness	Deep-set eyes	-	Bitemporal hollowing, hypoplastic midface	Abnormal nasal creases, loose skin folds	NR
Extremities								
Anomalies of the hands	Brachydactyly (bilateral)	Short fifth digits (bilateral)	–	–	–	Non-congenital syndactyly	NR	NR
Anomalies of the feet	Syndactyly of the second and third toes (bilateral)	Syndactyly of the second and third toes (bilateral)	–	–	–	Non-congenital syndactyly	NR	NR
Neurological								
Hypotonia	+	–	–	–	–	+	+	NR
Unstable gait	+	–	–	–	–	+	NR	NR
Ophthalmological								
Refractive error	Hypermetropia, astigmatism	Myopia, astigmatism	+ (type unknown)	Not tested	Hyperopia	–	NR	NR
Strabismus	–	–	–	Not tested	–	NR	NR	NR
Other	Nystagmus	–	–	–	–	NR	NR	Abnormal optic nerve, increased intraocular pressure
Dental								
Dental anomalies	–	–	–	Overbite	–	Atypical teeth	Neonatal teeth	NR
Immune system function								
Frequent infection	+	–	–	–	–	Low TREC at birth	No TREC at birth	NR

(Continued)

TABLE 1 | Continued

	Patient A	Patient B	Patient C	Patient D	Goos et al. (29)	Lessel et al. (28) (E:II-1)	Punwani et al. (27)	Longoni et al. (30) (T45)
Allergy/asthma	Asthma (light), allergy for HDM and grass pollen	–	–	–	–	+	NR	NR
Other								
Other features	Hypothyroidism Bicornate uterus	Proportionate short stature with delayed bone age	–	–	One epileptic episode	Mildly dilated aorta, severe obstructive sleep apnea due to micrognathia	Wormian skull bones, multiple brain anomalies on MRI (e.g., absent corpus callosum), umbilical hernia, mild pulmonary artery stenosis spastic quadriplegia and seizures	Scoliosis

Description of phenotypical and genetic information regarding our patients as well as previously reported patients. The patients reported by Goos et al. (32), Lessel et al. (28), and Punwani et al. (27) carried variants NM_138576.3(BCL11B):c.7C>A, p.(Arg3Ser), NM_138576.3(BCL11B): c.2421C>G, p.(Asn807Lys), and NM_138576.3(BCL11B): c.1323T>G, p.(Asn441Lys), respectively. The patient reported by Longoni et al. (30) carried a variant of BCL11B (BCL11B:NM_138576:exon4:c.C1048T: p.(Arg350Cys).

**, According to NM_138576; **, expression of variant verified in RNA extracted from peripheral blood lymphocyte; HDM, house dust mite, TREC, T-cell-receptor excision circle; NR, not reported; ‡, Combined Annotation Dependent Depletion, GRCh37-V1.6 (33, 34).*

CDH (30). All cases summarized above have missense variants in exon 4 with the exception of the patient reported by Goos et al. (29), who had a *de novo* missense mutation of exon 1.

DISCUSSION

In this study, we present four new patients with missense variants in *BCL11B* (Patient A: p.(Gly667Glu); Patient B: p.(Gly582Ser); Patient C: p. (Pro673Arg); Patient D: p.(Pro422Leu). Patient A displayed both CDH as well as craniosynostosis of the sagittal suture. The other three patients were diagnosed with craniosynostosis without a diaphragm defect. To our knowledge, this is the second study to report on craniosynostosis in patients with *BCL11B* mutations and the first to report on the co-occurrence of CDH and craniosynostosis in a patient with a missense *BCL11B* variant. Based on our current cohort of patients with BCL11B missense variants, the phenotype associated with *BCL11B* mutations is highly variable. Clinical features range from isolated craniosynostosis to CDH, severe immunological deficiencies and neurodevelopmental delays (**Table 1**).

BCL11B has a key function in fetal development and is involved in a multitude of systems and pathways (35). In line with this, many patients with BCL11B missense mutations are reported to suffer from a wide array of clinical anomalies (27, 28). In addition to altered craniofacial development, mutations, mostly loss of function mutations in BCL11B, have been reported to affect neurodevelopment as well as the development of the immune system, skin and the teeth (27–29, 36–49). In mice models, BCL11B is key in regulating suture patency, with a single disrupted allele causing synostosis (36). Goos et al. further investigated *BCL11B* variants in relation to craniosynostosis (29). A mouse model confirmed that the *de novo BCL11B* missense mutation (p.Arg3Ser) in their patient could have a causative effect on the development of craniosynostosis. Furthermore, they found several rare variants in a British cohort of craniosynostosis patients. However, these variants were dismissed as polymorphisms because of low impact or because they were inherited from a healthy parent. The authors suggest that only a specific subset of *BCL11B* mutations may cause craniosynostosis.

Our patients inherited the *BCL11B* variants from phenotypically normal parents. This could suggest incomplete penetrance, which may be similar to other craniosynostosis syndromes such as TCF12 and SMAD 6-related craniosynostosis and could potentially complicate genetic counseling (32, 50–52). Notably, patients reported in previous *BCL11B* studies mainly were carriers of *de novo* mutations. Although Combined Annotation Dependent Depletion (CADD) scores in our patients vary, patient A, C and D are in the same range as CADD scores of variants previously reported (27–29, 33, 34). Although a low CADD score could potentially indicate a benign variant, it is remarkable that four patients have now presented with craniosynostosis bearing a missense variant in exon 4 of *BCL11B.* Expression of this missense variant in blood excluded non-sense mediated RNA decay in patients A and B. Hence a dominant

TABLE 2A | Overview of disorders characterized by co-occurrence of craniosynostosis and congenital diaphragmatic hernia.

Clinical disorders with autosomal dominant inheritance pattern

	Gene (MIM number)	Phenotype MIM number	Chromosome	CS*	CDH‡	Key clinical features	Authors reporting on CDH and/or CS
Apert syndrome	FGFR2 (176943)	101200	10q26.13	Key feature, multisutural, progressive	Rare (six cases of CDH and one case of diaphragm agenesis)	– Symmetric syndactyly of hands and feet – Midface hypoplasia	Kaur 2019 Dap 2019 Kosinski 2016 Sobaih 2015 Bulfamante 2011 Wallis-Crespo 2004 Witters 2000
Kabuki syndrome (focus on type 1)	KMT2D (602113)	147920	12q13.12	Occasional	Relatively common	– Characteristic facial features: long palpebral fissures, everted lower eyelids, ptosis, arched eyebrows, blue sclera, cupped ears, micrognathia – Short stature, microcephaly – Intellectual disability (mild to moderate) – High/cleft palate and dental anomalies – Brachydactyly, clinodactyly, persistent fetal pads – Cardiac anomalies	Scott 2021 Topa 2017 Martinez-Lopez 2010 David 2004 Geneviève 2004 Van Haelst 2000
CEBALID** syndrome (MN1 C-terminal truncation syndrome)	MN1 (156100)	618774	22q12.1	Reported in three patients out of 25 identified patients identified to date	Reported in two patients out of 25 identified patients identified to date	– Characteristic facial features: midface hypoplasia, downslanting palpebral fissures, hypertelorism, exophthalmia, low-set ears, a short upturned nose – Intellectual disability, hypotonia, delay in motor development – Hearing loss – Structural brain anomalies	Mak 2020
Chromosome 22q11.2 deletion syndrome	-	145410; 188400; 192430; 600594; 601279; 601755; 602054; 609030	22q11.2	Rare feature (*may include CDC45 pathogenic variant in remaining allele*	Rare feature	– Highly variable phenotype (ranging from minor abnormities to major structural defects) – Cardiovascular anomalies – Cleft palate – Cognitive impairment – Short stature – Characteristic facial features: hypoplastic nasal alae, wide nasal bridge, short palpebral fissures, low-set, small ears – Nasal speech	Unolt 2020, 2017 McDonal-McGinn 2005
SPECC1L- related syndromes	SPECC1L (614140)	145410 145420 600251	22q11.23	Occasional	Occasional occurrence	– Characteristic facial features: hypertelorism, a wide, short nose, ptosis and retrognathia – Cleft lip/palate – Clinical features include branchial fistulas, omphalocele, genitourinary anomalies	Wild 2020 Bhoj 2019 Kruszka 2015 Robin 1995

(Continued)

TABLE 2A | Continued

Clinical disorders with autosomal dominant inheritance pattern							
	Gene (MIM number)	**Phenotype MIM number**	**Chromosome**	**CS***	**CDH‡**	**Key clinical features**	**Authors reporting on CDH and/or CS**
7q11. 23 Duplication syndrome	-	609757	7q11.23	Rare	Rare	– Variable expression, with incomplete penetrance – Characteristic facial features: prominent forehead, hypertelorism, high and broad nose, straight eyebrows, and thin lips – Cognitive impairment and intellectual disability – Epilepsy	Morris 2015 Van der Aa 2009 Torniero 2008 Kriek 2006
X-Linked							
Craniofrontonasal syndrome (XLD)	EFNB1 (300035)	304110	Xq13.1	Common feature, often either unilateral or bilateral coronal CS	Relatively common/ occasional	– More severe phenotype in females – Characteristic facial features: hypertelorism, craniofacial asymmetry, webbed neck, bifid tip of the nose, a broad nasal bridge – Clinodactyly of ≥ 1 digit – Longitudinal splitting/ridging of nails	Hogue 2010 Kawamoto 2007 Vasudevan 2006 Twigg 2004 & 2006 Brooks 2002 McGaughran 2002 Hurst 1988 Morris 1987
Cornelia de Lange syndrome	NIPBL (608667, AD) SMC1A (300040, XLD)	122470 300590	5p13.2 Xp11.22	Described for NIPBL variant; and for SMCA1	Key feature	– Characteristic facial features: thick, arched eyebrows or synophrys, long/smooth philtrum, short nose, thin upper vermillion – Limb defects – Intellectual disability – Growth retardation – Hirsutism	Desai 2021 Xu 2018 Gupta 2020
Simpson-Golabi-Behmel syndrome, Type 1 (XLR)	GPC3 (300037)	312870	Xq26.2	3 case reports	Occasional	– Characteristic facial features: hypertelorism, downslanting palpebral fissures – Cleft palate/lip. – Overgrowth and macrocephaly – Intellectual disability – Cardiac anomalies – Renal abnormality – Brachy-, syn-, and polydactyly	Schirwani 2019 Villarreal 2013 Li 2009

*This table presents an overview of clinical disorders in which both craniosynostosis and congenital diaphragmatic hernia have been reported more than once. *CS, craniosynostosis; ‡CDH, congenital diaphragmatic hernia; ** CEBALID, craniofacial defects, dysmorphic ears, structural brain abnormalities, expressive language delay, and impaired intellectual development; XLD, X-linked dominant; XLR, X-linked recessive.*

TABLE 2B | Isolated case reports on the co-occurrence of craniosynostosis and congenital diaphragmatic hernia.

	Gene (MIM number)	Phenotype MIM number	Chromosome	CS*	CDH‡	Key clinical features	Authors reporting on CDH and/or CS
Saethre -Chotzen	TWIST1 (601622)	101400	7p21.1	Key feature, often bicoronal CS	One case, unclear if co-occurrence of CDH is coincidental. Mouse models suggest a possible role for TWIST1 in development of the diaphragm	– Characteristic facial features: ptosis, downward slanting palpebral fissure, depressed nasal bridge, facial asymmetry – Small ears with prominent crus – Syndactyly of hand and feet	Piard 2012
Chromosome 9p deletion syndrome	-	158170	9p	Key feature: metopic CS	One case described for 9p deletion syndrome	– Characteristic facial features: hypotelorism, upslanting palpebral fissures, low-set ears, malformed ears, long philtrum – Moderate to severe intellectual disability	Alfi 1973
15q24 deletion syndrome	-	613406	15q24.2	1 case report	Four reports	– Characteristic facial features: high forehead, facial asymmetry, downslanting of eyes, hypertelorism, and a long smooth philtrum, ear malformations – Intellectual disability – Genitourinary anomalies – Cardiovascular malformations	Ng 2011 Van Esch 2009 Sharp 2007 Bettelheim 1998
DPF2-related Coffin–Siris syndrome	DPF2 (601671)	618027	11q12.1	At least two out of a total of 10 reported patients (one patient was stated to have trigonocephaly but no x-ray was performed) b	One patient described out of a total of 10 reported patients.	– Cognitive impairment, intellectual disability, and behavioral problems – Feeding problems and hypotonia – Hearing loss – Brachydactyly, clinodactyly, hypoplastic nails – Coarse facial features	Knapp 2019 Vasileiou 2018
-	DSC2 (125645)	-		One report of a patient with multisutural CS and CDH	One report of a patient with multisutural CS and CDH	Isolated case: presented with left atrial isomerism, transposed systemic and pulmonary veins, intestinal malrotation, bilateral inguinal hernia, hydronephrosis and nephrolithiasis in addition to CDH and CS	Das 2019
Loeys-Dietz syndrome	TGFBR1 (190181) TGFBR2 (190182)	609192 610168	9q22.33 3p24.1	Multiple cases reported	One report	– Aortic and arterial aneurysms – Characterstic facial features: hypertelorism, downslant of the eyes – Cleft palate, bifid uvula – Pectus anomalies – Arachnodactyly	Lobaton 2021 Loeys 2005
Gain of function of RARB	RARB			One report of a patient with CS	Multiple patients with diaphragmatic hernia	Thirteen cases have been reported in total. Clinical features include microphthalmia and anophthalmia, sclerocornea, and coloboma, as well as cardiac anomalies, and malrotation of the bowel	Srour 2016

**CS, craniosynostosis; ‡CDH, congenital diaphragmatic hernia.*

negative effect or an altered function of the mutated protein could be an explanation of the more severe phenotype.

To our knowledge, only one previous study reported on a patient with a CDH with a *BCL11B* missense mutation [p.(Arg350Cys); CADD score: 31] (30). We now report on a second patient with a *BCL11B* missense variant in exon 4 with CDH. Although further studies remain necessary to assess if *BCL11B* is a causative factor in this CDH phenotype the fact that *BCL11B* is involved in pathways that overlap with pathways that have been previously linked to CDH support this observation. *BCL11B* likely interacts with a CDH causative gene (NR2F2) as well as with other CDH candidate genes (CREBBP, EP300, CHD4) (1, 53). The genetic etiologies of both CDH and craniosynostosis are highly complex and definitive genetic pathways remain to be further elucidated. Although the combination of CDH and craniosynostosis is rare, the fact that multiple syndromes are associated with both craniosynostosis and CDH suggests a possible overlap between craniosynostosis and CDH pathways. For instance, NR2F2, a causative CDH gene with which *BCL11B* has been shown to interact (54, 55), appears to have a function in mesenchymal cell differentiation in embryogenesis including a regulatory function in myogenesis, chondrogenesis, and osteogenesis (56). These processes are disturbed in CDH and craniosynostosis. NR2F2 also regulates Runx2, which has been reported to be overexpressed in some types of craniosynostosis, and has a function in the retinoic acid signaling pathway regulation, which is key in the development of CDH (1, 56–61). Further studies are needed to establish definitive CDH and craniosynostosis pathways and to assess if these pathways are interlinked.

The phenotype of our patients is similar to previously reported clinical phenotypes of patients with *BCL11B* missense mutations, which included craniosynostosis and CDH (6, 27, 28). Therefore, it is implied that both CDH and craniosynostosis may be features associated with *BCL11B* missense mutations. Further functional studies are required to assess if these variants are coincidental findings or if they indeed have a causative effect on craniosynostosis and CDH.

Patients with missense mutations in *BCL11B* appear to be affected more severely than patients with loss of function or other types of *BCL11B* mutations (28). In a previous study, the most severely affected patient carried a missense mutation in a zinc-finger domain in exon 4 (patient EII-I, **Table 1**) (28). *BCL11B* encodes for a zing finger protein transcription factor and function both as a transcriptional activator as well as a repressor (35, 62). Lessel et al. hypothesized that these missense mutations may not only lead to a loss of DNA binding but also to novel DNA binding sites in other genes (28). Similar observations were made in a previous study, which demonstrated that a mutation p.(Asn441Lys) led to both decreased binding of BCL11B to original target DNA sites as well as to the promotion of novel target DNA binding sites (27). We hypothesize that also these BCL11B missense variants may either have a dominant negative effect or induce new target genes, thereby causing a more severe phenotype in patients with these missense variants /mutations as compared to eg loss of function type of mutations. Future studies should further examine the *in vivo* effect of these specific *BCL11B* mutations in animal models to establish if the described variants are indeed disease causing mutants or coincidental findings, as shown by Goos et al. for the mutation in their patient (29).

Although we are the first to report on the co-occurrence of CDH and craniosynostosis in a patient with a *BCL11B* missense mutation, the co-occurrence of CDH and craniosynostosis has been described previously for several syndromes, such as Apert's syndrome and craniofrontonasal syndrome (7–22). Craniosynostosis syndromes comprise ∼30% of all craniosynostosis cases (63). Syndromic craniosynostosis is highly heterogeneous and is often associated with extracranial anomalies, including neurologic, limb, ophthalmologic and cardiac anomalies. Most craniosynostosis syndromes have an autosomal dominant inheritance pattern, although many cases arise from *de novo* mutations (32, 64). We conducted a literature search to identify which craniosynostosis syndromes are associated with CDH. We found nine syndromes to include both CDH as well as craniosynostosis as an associated feature, based on searches in OMIM, Medline, Science Direct and a gray literature search in Google Scholar. **Table 2A** summarizes the genetic anomalies as well as the main features of each syndrome reported to include both craniosynostosis as well as CDH. In addition, we found seven isolated case reports that describe patients presenting with both craniosynostosis as well as CDH, which are shown in **Table 2B**. The supplemental information includes **Table 2** with a full reference list. Although CDH appears to be a rare feature in craniosynostosis syndromes, craniosynostosis is often treatable with surgical intervention and has high survival rates. In contrast, CDH is a potentially lethal disorder and is associated strongly with poor long-term clinical outcome (66–68). Awareness of this rare feature in craniosynostosis syndromes therefore is key.

CONCLUSION

This report implies that both CDH as well as craniosynostosis are features of *BCL11B* missense mutations. However, further studies are required to establish if *BCL11B* missense mutations are indeed a causative factor or if our finding was coincidental.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained

from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

LG, AK, IM, and MD contributed to the study's conception, wrote the manuscript, and provided critical revisions. LG, AG, and MD assessed the clinical and phenotypical features of the patients. FM and FJ assisted with data collection and provided genetic information. AG, QB, DV, and EB provided critical revision. All authors approved the article for submission.

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SUPPLEMENTARY MATERIAL

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REFERENCES

1. Kammoun M, Souche E, Brady P, Ding J, Cosemans N, Gratacos E, et al. Genetic profile of isolated congenital diaphragmatic hernia revealed by targeted next-generation sequencing. *Prenat Diagn.* (2018) 38:654–63. doi: 10.1002/pd.5327
2. McGivern MR, Best KE, Rankin J, Wellesley D, Greenlees R, Addor MC, et al. Epidemiology of congenital diaphragmatic hernia in Europe: a register-based study. *Arch Dis Child Fetal Neonatal Ed.* (2015) 100:F137–44. doi: 10.1136/archdischild-2014-306174
3. Gallot D, Boda C, Ughetto S, Perthus I, Robert-Gnansia E, Francannet C, et al. Prenatal detection and outcome of congenital diaphragmatic hernia: a French registry-based study. *Ultrasound Obstet Gynecol.* (2007) 29:276–83. doi: 10.1002/uog.3863
4. Paoletti M, Raffler G, Gaffi MS, Antounians L, Lauriti G, Zani A. Prevalence and risk factors for congenital diaphragmatic hernia: a global view. *J Pediatr Surg.* (2020) 55:2297–307. doi: 10.1016/j.jpedsurg.2020.06.022
5. Zaiss I, Kehl S, Link K, Neff W, Schaible T, Sutterlin M, et al. Associated malformations in congenital diaphragmatic hernia. *Am J Perinatol.* (2011) 28:211–8. doi: 10.1055/s-0030-1268235
6. Robert E, Kallen B, Harris J. The epidemiology of diaphragmatic hernia. *Eur J Epidemiol.* (1997) 13:665–73. doi: 10.1023/A:1007395727819
7. Kaur R, Mishra P, Kumar S, Sankar MJ, Kabra M, Gupta N. Apert syndrome with congenital diaphragmatic hernia: another case report and review of the literature. *Clin Dysmorphol.* (2019) 28:78–80. doi: 10.1097/MCD.0000000000000261
8. Bulfamante G, Gana S, Avagliano L, Fabietti I, Gentilin B, Lalatta F. Congenital diaphragmatic hernia as prenatal presentation of Apert syndrome. *Prenat Diagn.* (2011) 31:910–1. doi: 10.1002/pd.2788
9. Dap M, Bach-Segura P, Bertholdt C, Menzies D, Masutti JP, Klein O, et al. Variable phenotypic expression of Apert syndrome in monozygotic twins. *Clin Case Rep.* (2019) 7:54–7. doi: 10.1002/ccr3.1915
10. Kosinski P, Luterek K, Wielgos M. Diaphragmatic hernia as an early ultrasound manifestation of Apert syndrome. *Ginekol Pol.* (2016) 87:830. doi: 10.5603/GP.2016.0097
11. Sobaih BH, AlAli AA. A third report of Apert syndrome in association with diaphragmatic hernia. *Clin Dysmorphol.* (2015) 24:106–8. doi: 10.1097/MCD.0000000000000083
12. Wallis-Crespo MC, Gilbert-Barness E. Pathology teach and tell: acrocephalosyndactyly type I (Apert syndrome). *Fetal Pediatr Pathol.* (2004) 23:71–8. doi: 10.1080/15227950490423160
13. Witters I, Devriendt K, Moerman P, van Hole C, Fryns JP. Diaphragmatic hernia as the first echographic sign in Apert syndrome. *Prenat Diagn.* (2000) 20:404–6. doi: 10.1002/(SICI)1097-0223(200005)20:5<404::AID-PD813>3.0.CO;2-0
14. Brooks AS, van Dooren M, Hoogeboom J, Gischler S, Willems PJ, Tibboel D. Congenital diaphragmatic hernia in a female patient with craniofrontonasal syndrome. *Clin Dysmorphol.* (2002) 11:151–3. doi: 10.1097/00019605-200204000-00019
15. Hogue J, Shankar S, Perry H, Patel R, Vargervik K, Slavotinek A. A novel EFNB1 mutation (c.712delG) in a family with craniofrontonasal syndrome and diaphragmatic hernia. *Am J Med Genet Part A.* (2010) 152A:2574–7. doi: 10.1002/ajmg.a.33596
16. Hurst J, Baraitser M. Craniofrontonasal dysplasia. *J Med Genet.* (1988) 25:133–4. doi: 10.1136/jmg.25.2.133
17. Kawamoto HK, Heller JB, Heller MM, Urrego A, Gabbay JS, Wasson KL, et al. Craniofrontonasal dysplasia: a surgical treatment algorithm. *Plast Reconstr Surg.* (2007) 120:1943–56. doi: 10.1097/01.prs.0000287286.12944.9f
18. McGaughran J, Rees M, Battin M. Craniofrontonasal syndrome and diaphragmatic hernia. *Am J Med Genet.* (2002) 110:391–2. doi: 10.1002/ajmg.10176
19. Morris CA, Palumbos JC, Carey JC. Delineation of the male phenotype in carniofrontonasal syndrome. *Am J Med Genet.* (1987) 27:623–31. doi: 10.1002/ajmg.1320270315
20. Twigg SR, Kan R, Babbs C, Bochukova EG, Robertson SP, Wall SA, et al. Mutations of ephrin-B1 (EFNB1), a marker of tissue boundary formation, cause craniofrontonasal syndrome. *Proc Natl Acad Sci USA.* (2004) 101:8652–7. doi: 10.1073/pnas.0402819101
21. Twigg SR, Matsumoto K, Kidd AM, Goriely A, Taylor IB, Fisher RB, et al. The origin of EFNB1 mutations in craniofrontonasal syndrome: frequent somatic mosaicism and explanation of the paucity of carrier males. *Am J Hum Genet.* (2006) 78:999–1010. doi: 10.1086/504440
22. Vasudevan PC, Twigg SR, Mulliken JB, Cook JA, Quarrell OW, Wilkie AO. Expanding the phenotype of craniofrontonasal syndrome: two unrelated boys with EFNB1 mutations and congenital diaphragmatic hernia. *Eur J Hum Genet.* (2006) 14:884–7. doi: 10.1038/sj.ejhg.5201633
23. Cornelissen M, Ottelander B, Rizopoulos D, van der Hulst R, Mink van der Molen A, van der Horst C, et al. Increase of prevalence of craniosynostosis. *J Craniomaxillofac Surg.* (2016) 44:1273–9. doi: 10.1016/j.jcms.2016.07.007
24. de Jong T, Bannink N, Bredero-Boelhouwer HH, van Veelen ML, Bartels MC, Hoeve LJ, et al. Long-term functional outcome in 167 patients with syndromic craniosynostosis; defining a syndrome-specific risk profile. *J Plast Reconstr Aesthet Surg.* (2010) 63:1635–41. doi: 10.1016/j.bjps.2009.10.029
25. Maliepaard M, Mathijssen IM, Oosterlaan J, Okkerse JM. Intellectual, behavioral, and emotional functioning in children with syndromic craniosynostosis. *Pediatrics.* (2014) 133:e1608–15. doi: 10.1542/peds.2013-3077
26. Mathijssen IMJ, Craniosynostosis Working Group. Guideline. Updated guideline on treatment and management of craniosynostosis. *J Craniofac Surg.* (2021) 32:371–450. doi: 10.1097/SCS.0000000000007035
27. Punwani D, Zhang Y, Yu J, Cowan MJ, Rana S, Kwan A, et al. Multisystem anomalies in severe combined immunodeficiency with mutant BCL11B. *N Engl J Med.* (2016) 375:2165–76. doi: 10.1056/NEJMoa1509164
28. Lessel D, Gehbauer C, Bramswig NC, Schluth-Bolard C, Venkataramanappa S, van Gassen KLI, et al. BCL11B mutations in patients affected by a neurodevelopmental disorder with reduced type 2 innate lymphoid cells. *Brain.* (2018) 141:2299–311. doi: 10.1093/brain/awy173
29. Goos JAC, Vogel WK, Mlcochova H, Millard CJ, Esfandiari E, Selman WH, et al. A *de novo* substitution in BCL11B leads to loss of interaction with

transcriptional complexes and craniosynostosis. *Hum Mol Genet.* (2019) 28:2501–13. doi: 10.1093/hmg/ddz072
30. Longoni M, High FA, Qi H, Joy MP, Hila R, Coletti CM, et al. Genome-wide enrichment of damaging *de novo* variants in patients with isolated and complex congenital diaphragmatic hernia. *Hum Genet.* (2017) 136:679–91. doi: 10.1007/s00439-017-1774-y
31. Tweedie S, Braschi B, Gray K, Jones TEM, Seal RL, Yates B, et al. Genenames.org: the HGNC and VGNC resources in 2021. *Nucleic Acids Res.* (2021) 49:D939–46. doi: 10.1093/nar/gkaa980
32. Goos JAC, Mathijssen IMJ. Genetic causes of craniosynostosis: an update. *Mol Syndromol.* (2019) 10:6–23. doi: 10.1159/000492266
33. CADD. © *University of Washington Hudson-Alpha Institute for Biotechnology and Berlin Institute of Health. Combined Annotation Dependent Depletion; Single Nucleotide Variant (SNV) Lookup.* (2021). Available online at: https://cadd.gs.washington.edu/snv
34. Rentzsch P, Schubach M, Shendure J, Kircher M. CADD-Splice-improving genome-wide variant effect prediction using deep learning-derived splice scores. *Genome Med.* (2021) 13:31. doi: 10.1186/s13073-021-00835-9
35. Lennon MJ, Jones SP, Lovelace MD, Guillemin GJ, Brew BJ. Bcl11b-A critical neurodevelopmental transcription factor-roles in health and disease. *Front Cell Neurosci.* (2017) 11:89. doi: 10.3389/fncel.2017.00089
36. Kyrylkova K, Iwaniec UT, Philbrick KA, Leid M. BCL11B regulates sutural patency in the mouse craniofacial skeleton. *Dev Biol.* (2016) 415:251–60. doi: 10.1016/j.ydbio.2015.10.010
37. Wakabayashi Y, Watanabe H, Inoue J, Takeda N, Sakata J, Mishima Y, et al. Bcl11b is required for differentiation and survival of alphabeta T lymphocytes. *Nat Immunol.* (2003) 4:533–9. doi: 10.1038/ni927
38. Li L, Leid M, Rothenberg EV. An early T cell lineage commitment checkpoint dependent on the transcription factor Bcl11b. *Science.* (2010) 329:89–93. doi: 10.1126/science.1188989
39. Albu DI, VanValkenburgh J, Morin N, Califano D, Jenkins NA, Copeland NG, et al. Transcription factor Bcl11b controls selection of invariant natural killer T-cells by regulating glycolipid presentation in double-positive thymocytes. *Proc Natl Acad Sci USA.* (2011) 108:6211–6. doi: 10.1073/pnas.1014304108
40. Arlotta P, Molyneaux BJ, Jabaudon D, Yoshida Y, Macklis JD. Ctip2 controls the differentiation of medium spiny neurons and the establishment of the cellular architecture of the striatum. *J Neurosci.* (2008) 28:622–32. doi: 10.1523/JNEUROSCI.2986-07.2008
41. Golonzhka O, Liang X, Messaddeq N, Bornert JM, Campbell AL, Metzger D, et al. Dual role of COUP-TF-interacting protein 2 in epidermal homeostasis and permeability barrier formation. *J Invest Dermatol.* (2009) 129:1459–70. doi: 10.1038/jid.2008.392
42. Golonzhka O, Metzger D, Bornert JM, Bay BK, Gross MK, Kioussi C, et al. Ctip2/Bcl11b controls ameloblast formation during mammalian odontogenesis. *Proc Natl Acad Sci USA.* (2009) 106:4278–83. doi: 10.1073/pnas.0900568106
43. Kastner P, Chan S, Vogel WK, Zhang LJ, Topark-Ngarm A, Golonzhka O, et al. Bcl11b represses a mature T-cell gene expression program in immature CD4(+)CD8(+) thymocytes. *Eur J Immunol.* (2010) 40:2143–54. doi: 10.1002/eji.200940258
44. Simon R, Baumann L, Fischer J, Seigfried FA, De Bruyckere E, Liu P, et al. Structure-function integrity of the adult hippocampus depends on the transcription factor Bcl11b/Ctip2. *Genes Brain Behav.* (2016) 15:405–19. doi: 10.1111/gbb.12287
45. Simon R, Brylka H, Schwegler H, Venkataramanappa S, Andratschke J, Wiegreffe C, et al. A dual function of Bcl11b/Ctip2 in hippocampal neurogenesis. *EMBO J.* (2012) 31:2922–36. doi: 10.1038/emboj.2012.142
46. Uddin MN, Zhang Y, Harton JA, MacNamara KC, Avram D. TNF-alpha-dependent hematopoiesis following Bcl11b deletion in T cells restricts metastatic melanoma. *J Immunol.* (2014) 192:1946–53. doi: 10.4049/jimmunol.1301976
47. Vanvalkenburgh J, Albu DI, Bapanpally C, Casanova S, Califano D, Jones DM, et al. Critical role of Bcl11b in suppressor function of T regulatory cells and prevention of inflammatory bowel disease. *J Exp Med.* (2011) 208:2069–81. doi: 10.1084/jem.20102683
48. Kyrylkova K, Kyryachenko S, Biehs B, Klein O, Kioussi C, Leid M. BCL11B regulates epithelial proliferation and asymmetric development of the mouse mandibular incisor. *PLoS ONE.* (2012) 7:e37670. doi: 10.1371/journal.pone.0037670
49. Albu DI, Feng D, Bhattacharya D, Jenkins NA, Copeland NG, Liu P, et al. BCL11B is required for positive selection and survival of double-positive thymocytes. *J Exp Med.* (2007) 204:3003–15. doi: 10.1084/jem.20070863
50. Timberlake AT, Choi J, Zaidi S, Lu Q, Nelson-Williams C, Brooks ED, et al. Two locus inheritance of non-syndromic midline craniosynostosis via rare SMAD6 and common BMP2 alleles. *Elife.* (2016) 5:e20125. doi: 10.7554/eLife.20125
51. Sharma VP, Fenwick AL, Brockop MS, McGowan SJ, Goos JA, Hoogeboom AJ, et al. Mutations in TCF12, encoding a basic helix-loop-helix partner of TWIST1, are a frequent cause of coronal craniosynostosis. *Nat Genet.* (2013) 45:304–7. doi: 10.1016/S0140-6736(13)60554-1
52. di Rocco F, Baujat G, Arnaud E, Renier D, Laplanche JL, Daire VC, et al. Clinical spectrum and outcomes in families with coronal synostosis and TCF12 mutations. *Eur J Hum Genet.* (2014) 22:1413–6. doi: 10.1038/ejhg.2014.57
53. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res.* (2019) 47:D607–13. doi: 10.1093/nar/gky1131
54. Sidwell T, Rothenberg EV. Epigenetic dynamics in the function of t-lineage regulatory factor Bcl11b. *Front Immunol.* (2021) 12:669498. doi: 10.3389/fimmu.2021.669498
55. Avram D, Fields A, Pretty On Top K, Nevrivy DJ, Ishmael JE, Leid M. Isolation of a novel family of C(2)H(2) zinc finger proteins implicated in transcriptional repression mediated by chicken ovalbumin upstream promoter transcription factor (COUP-TF) orphan nuclear receptors. *J Biol Chem.* (2000) 275:10315–22. doi: 10.1074/jbc.275.14.10315
56. Xie X, Qin J, Lin SH, Tsai SY, Tsai MJ. Nuclear receptor chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII) modulates mesenchymal cell commitment and differentiation. *Proc Natl Acad Sci USA.* (2011) 108:14843–8. doi: 10.1073/pnas.1110236108
57. Cuellar A, Bala K, Di Pietro L, Barba M, Yagnik G, Liu JL, et al. Gain-of-function variants and overexpression of RUNX2 in patients with nonsyndromic midline craniosynostosis. *Bone.* (2020) 137:115395. doi: 10.1016/j.bone.2020.115395
58. Lee KN, Jang WG, Kim EJ, Oh SH, Son HJ, Kim SH, et al. Orphan nuclear receptor chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII) protein negatively regulates bone morphogenetic protein 2-induced osteoblast differentiation through suppressing runt-related gene 2 (Runx2) activity. *J Biol Chem.* (2012) 287:18888–99. doi: 10.1074/jbc.M111.311878
59. Kruse SW, Suino-Powell K, Zhou XE, Kretschman JE, Reynolds R, Vonrhein C, et al. Identification of COUP-TFII orphan nuclear receptor as a retinoic acid-activated receptor. *PLoS Biol.* (2008) 6:e227. doi: 10.1371/journal.pbio.0060227
60. Kardon G, Ackerman KG, McCulley DJ, Shen Y, Wynn J, Shang L, et al. Congenital diaphragmatic hernias: from genes to mechanisms to therapies. *Dis Model Mech.* (2017) 10:955–70. doi: 10.1242/dmm.028365
61. Clugston RD, Zhang W, Alvarez S, de Lera AR, Greer JJ. Understanding abnormal retinoid signaling as a causative mechanism in congenital diaphragmatic hernia. *Am J Respir Cell Mol Biol.* (2010) 42:276–85. doi: 10.1165/rcmb.2009-0076OC
62. Kominami R. Role of the transcription factor Bcl11b in development and lymphomagenesis. *Proc Jpn Acad Ser B Phys Biol Sci.* (2012) 88:72–87. doi: 10.2183/pjab.88.72
63. Wilkie AOM, Johnson D, Wall SA. Clinical genetics of craniosynostosis. *Curr Opin Pediatr.* (2017) 29:622–8. doi: 10.1097/MOP.0000000000000542
64. Twigg SR, Wilkie AO. A genetic-pathophysiological framework for craniosynostosis. *Am J Hum Genet.* (2015) 97:359–77. doi: 10.1016/j.ajhg.2015.07.006
65. Srour M, Caron V, Pearson T, Nielsen SB, Levesque S, Delrue MA, et al. Gain-of-function mutations in RARB cause intellectual disability with progressive motor impairment. *Hum Mutat.* (2016) 37:786–93. doi: 10.1002/humu.23004

66. Congenital Diaphragmatic Hernia Study G, Lally KP, Lally PA, Lasky RE, Tibboel D, Jaksic T, et al. Defect size determines survival in infants with congenital diaphragmatic hernia. *Pediatrics.* (2007) 120:e651–7. doi: 10.1542/peds.2006-3040
67. Coughlin MA, Werner NL, Gajarski R, Gadepalli S, Hirschl R, Barks J, et al. Prenatally diagnosed severe CDH: mortality and morbidity remain high. *J Pediatr Surg.* (2016) 51:1091–5. doi: 10.1016/j.jpedsurg.2015.10.082
68. Chatterjee D, Ing RJ, Gien J. Update on congenital diaphragmatic hernia. *Anesth Analg.* (2020) 131:808–21. doi: 10.1213/ANE.0000000000004324

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Cellular Origin(s) of Congenital Diaphragmatic Hernia

Gabriëla G. Edel[1,2], *Gerben Schaaf*[3,4,5†], *Rene M. H. Wijnen*[1†], *Dick Tibboel*[1†], *Gabrielle Kardon*[6†] *and Robbert J. Rottier*[1,2*†]

[1] Department of Pediatric Surgery and Intensive Care, Erasmus MC-Sophia Children's Hospital, Rotterdam, Netherlands, [2] Department of Cell Biology, Erasmus MC, Rotterdam, Netherlands, [3] Department of Clinical Genetics, Erasmus MC, Rotterdam, Netherlands, [4] Department of Pediatrics, Erasmus MC-Sophia Children's Hospital, Rotterdam, Netherlands, [5] Center for Lysosomal and Metabolic Diseases, Erasmus MC, Rotterdam, Netherlands, [6] Department of Human Genetics, University of Utah, Salt Lake City, UT, United States

Congenital diaphragmatic hernia (CDH) is a structural birth defect characterized by a diaphragmatic defect, lung hypoplasia and structural vascular defects. In spite of recent developments, the pathogenesis of CDH is still poorly understood. CDH is a complex congenital disorder with multifactorial etiology consisting of genetic, cellular and mechanical factors. This review explores the cellular origin of CDH pathogenesis in the diaphragm and lungs and describes recent developments in basic and translational CDH research.

Keywords: congenital diaphragmatic hernia (CDH), diaphragm, pleuroperitoneal folds, perivascular cells, mesothelium

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****Correspondence:***
Robbert J. Rottier
r.rottier@erasmusmc.nl

†ORCID:
Gerben Schaaf
orcid.org/0000-0003-0189-9073
Rene M. H. Wijnen
orcid.org/0000-0001-7266-9713
Dick Tibboel
orcid.org/0000-0003-4331-5398
Gabrielle Kardon
orcid.org/0000-0003-2144-4463
Robbert J. Rottier
orcid.org/0000-0002-9291-4971

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INTRODUCTION

In the past 25 years, the general mortality rate of CDH has decreased to approximately 25%, but the mortality rate remained 50% in patients who receive extracorporeal membrane oxygenation (ECMO) (1, 2). In a recent study, CDH patients were categorized pre-ECMO in a low-risk, moderate-risk or high-risk cohort by their risk score (RS) for mortality, which is based on multiple risk factors, like location of the hernia and weight before ECMO (1). Change in individual likelihood of death overtime was different for each cohort: it was increased in the low risk group, decreased in the moderate risk group and unchanged in the high-risk group. Although the average survival of CDH patients increased in the past decades as a result of advancements in prenatal diagnosis, CDH pathogenesis remains still poorly understood. The main reason is that CDH is a complex congenital disorder with multifactorial etiology including genetic, cellular and environmental factors (3–5). In this review, we will discuss different tissues and cell types that are implicated in CDH pathogenesis and review recent developments in basic and translational research in CDH.

DIAPHRAGM/PPF

The diaphragm is an essential muscle that is critical for proper respiration and forms a barrier between the thoracic and abdominal cavities (6). The diaphragm develops from multiple embryonic sources. Of primary importance are the pleuroperitoneal folds (PPFs). The PPFs are paired transient pyramidal-shaped structures located between the thoracic (pleural) and abdominal (peritoneal) cavities. The PPFs expand dorsally and ventrally across the cranial surface of the liver to give rise to the diaphragm's muscle connective tissue and central tendon (7–9). The somites, segmental structures lying adjacent to the neural tube, are the source of the diaphragm muscle.

Muscle progenitors emigrate from the cervical somites to the nascent PPFs and as the PPFs expand, the progenitors migrate and fuse into the radial array of costal myofibers (7–11). In addition, the diaphragm is innervated by nerves that arise from the C3-C5 segments of the neural tube and it is vascularized by endothelial cells derived from the somites and likely splanchnic lateral plate mesoderm (8–10).

The PPFs are essential for the morphogenesis of the diaphragm and defects in the PPFs lead to CDH. Experiments in mice genetically tracking the development of the PPFs have established that the expansion of the PPFs drives the overall morphogenesis of the diaphragm and guide diaphragm muscle development (7). Defects in the development of the PPFs are a significant source of CDH. Most CDH-implicated genes that have been examined are expressed in the PPF cells (7, 12–15). Mutations in CDH-implicated genes can lead to the incomplete expansion of the PPFs and thus lead to incompletely developed diaphragms that allow herniation of abdominal contents into thoracic cavity [e.g., (16)]. Alternatively, PPFs harboring mutations may be unable to signal to muscle progenitors [e.g., (7)], leading to defective progenitor migration to the PPFs, increased apoptosis, and/or decreased proliferation or differentiation into myofibers. In such scenarios, defects in the PPF cells lead to cell non-autonomous effects on neighboring muscle, resulting in weaker muscleless regions that allow herniation (7). To date, all evidence suggests that CDH arises from primary defects in the PPFs by either defective generation/migration of cell populations or impaired muscularization of the diaphragm, with little evidence to support a primary role for defects in muscle cells.

LUNG MESOTHELIUM

Pulmonary hypoplasia is another characteristic of CDH. Although a lower amount of alveolar type I cells have been identified in nitrofen-induced CDH (17, 18), the tissue that is primarily defective in CDH-associated hypoplasia is not yet clear. A potential cellular source in CDH pathogenesis is the lung mesothelium, which contributes to different cell populations in the lung and is important for proper mesenchymal growth. The pleural mesothelium is a monolayer of cells that forms a lining around the lungs and is derived from the embryonic mesoderm (19). Although mesothelial cells are mesenchymal in origin, they have epithelial characteristics (20). During development, pulmonary mesothelial cells (PMCs) undergo epithelial-mesenchymal transition and differentiate to contribute to different cell populations in the pulmonary mesenchyme under the influence of active hedgehog signaling (21).

Several studies reported conflicting data to what extent the embryonic mesothelium contributes to the pulmonary mesenchyme. These studies employed different *Wilm's tumor 1* (*Wt1*)-dependent driver lines, which is a gene encoding a zinc finger transcription factor expressed in mesothelial cells and diaphragm and that is associated with CDH (22). *Wt1*-dependent lines were used to trace $WT1^+$ mesothelial cells and analyzed the fate of the progeny at several time points, which most likely explains the variation in their results. Que et al. (23) reported that $WT1^+$ lung mesothelial cells contribute to vascular smooth muscle cells (SMCs) and to alveoli, which were potentially interstitial fibroblasts, alveolar myofibroblasts or endothelial cells. Dixit et al. chose a conditional strategy to lineage trace *Wt1*-expressing mesothelial cells and reported contribution of PMCs to vascular and bronchial smooth muscle cells, as well as fibroblasts (21). Cano et al. (24) focused on the contribution of PMCs to different lung cell types during embryonic lung development and observed contribution to a wider range of cell types including endothelial cells, airway and vascular smooth muscle cells, pulmonary cartilage and fibroblasts. Lastly, Von Gise et al. (25) found that labeling $WT1^+$ cells at E10.5 resulted in a small contribution to bronchial- and vascular smooth muscle cells, while the majority differentiated into $PDGFR\alpha^+$-fibroblasts or $PDGFR\beta^+/NG2^+$-pericytes. Von Gise et al. (25) also showed that only fetal and not postnatal PMCs are capable of differentiating into pulmonary mesenchymal cell types. Postnatal PMCs remain in the mesothelial lining and do not migrate out or differentiate into other cell types. As such, postnatal PMCs do not seem to contribute to the lung parenchyma during normal lung homeostasis or after injury (25). In conclusion, mesothelial cells contribute to the bronchial- and vascular smooth muscle cell population as well as to fibroblasts and pericytes and that this only occurs during embryonic development and not postnatally (21, 25).

Besides being a progenitor source during lung development, the mesothelium also acts as a signaling source. One important pathway during development of the lungs is the FGF signaling pathway, in which FGF9 and FGF10 are essential (26). FGF10 is expressed in the lung mesenchyme, while FGF9 is expressed in the mesothelium and epithelium, signaling to the submesothelial- and subepithelial mesenchyme, respectively (27, 28). Mesothelial- and epithelial-produced FGFs have a different function: mesothelial-derived FGF9 is mainly responsible for mesenchymal growth by maintaining mesenchymal FGF-WNT/β-catenin signaling, whereas epithelial-derived FGF9 influences epithelial branching (29). One study reported reduced pulmonary FGF9 expression in the nitrofen-induced CDH rat model (30). However, recombinant co-cultures of fibroblasts and epithelial cells of nitrofen-treated- and control rats showed that not epithelial cells, but fibroblasts, are defective in nitrofen-induced hypoplastic lungs, showing decreased apoptosis and increased proliferation (31). Since the mesothelium is a source for mesenchymal fibroblasts (21, 23–25) and expresses FGF9 for the regulation of mesenchymal growth (29), the mesothelium is a potential source for CDH-associated lung hypoplasia.

Cano et al. (24) showed that homozygous *Wt1* knock-out ($Wt1^{-/-}$) mice had a CDH-like phenotype, abnormally fused lung lobes and reduced immunoreactivity for FGF9 in the pulmonary mesenchyme and mesothelium. A recent study reported similar findings and also reported an aberrant lung branching architecture already before closure of the diaphragm, when WT1 is expressed (32). When $Wt1^{-/-}$ lungs were cultured *ex vivo*, lung branching was normal and any hypoplasia that had originated *in vivo*, was restored within 24 h *ex vivo* (32). Additional analyses showed that the space in the chest

cavity—that is usually present for the lungs to grow—was nearly absent, explaining why culturing the lungs *ex vivo* without physical constraints recovered branching (32). Aberrant WT1 expression in the lung mesothelium results in defective lung development and CDH as result of limited space in the chest cavity and potentially by defective signaling and migration of mesothelial cells.

In summary, the mesothelium acts as a progenitor source and signaling center for the pulmonary mesenchyme to facilitate proper mesenchymal growth and cellular differentiation. These results associate the lung mesothelium as a cellular contributor to CDH.

(PERI)VASCULAR CELLS

Besides a diaphragmatic defect and pulmonary hypoplasia, almost all CDH patients have pulmonary hypertension (33, 34), which is caused by an altered development of the pulmonary vasculature and pulmonary vascular remodeling (35). Changes in cell phenotypes, cellular proliferation and defective cell-cell communication have been proposed as underlying causes.

Previously, it was shown that CDH patients have higher abundance of contractile vascular SMCs, which were also more distributed along the proximo-distal axis of the lung vasculature (36). Although inhaled nitric oxide treatment is a successful treatment for preterm babies, it is only effective in a small number of CDH patients and even only beneficial in certain subsets of CDH patients (37). The pathological changes in vascular SMCs indicate a disturbed pulmonary vascular development and might explain the ineffectiveness of inhaled nitric oxide treatment in CDH patients. Another study by Acker et al. (38) showed increased proliferation of pulmonary arterial SMCs and pulmonary arterial SMC hyperplasia in a surgical CDH lamb model. This was not caused by an altered SMC phenotype, but by a disturbed interaction with pulmonary arterial endothelial cells (PAECs), indicating that defective endothelial signaling contributed to SMC hyperplasia and may therefore result in pulmonary hypertension (38). In the nitrofen-induced CDH mouse model, Kool et al. (39) observed an increased pericyte coverage in the large pulmonary vessels and pericytes had a more contractile phenotype. Furthermore, the basement membrane around the midsized vessels was discontinuous, indicating defective cross-talk between pericytes and endothelial cells (39). The impaired cross-talk between those two cell types and the altered pericyte phenotype may be the origin of pulmonary hypertension in CDH.

CDH patients show a decreased vascular growth that contributes to poor disease outcomes. Acker et al. (40) showed in the surgical CDH lamb model reduced proliferation and tube formation capacity of PAECs. They also found a marked reduction in high-proliferative PAECs, which is a progenitor subpopulation of endothelial cells (41, 42). A reduced capillary network was also observed by Kool et al. (39) in the nitrofen-induced CDH mouse model. These results suggest that reduced proliferation of endothelial cells contributes to the decreased vascular growth in CDH patients.

Altogether, several cell types may disturb the lung vascular network, leading to CDH-associated phenotypes. Abnormal phenotypes of endothelial cells, SMCs and pericytes and defective interactions between them may be the basis for the simplified vascular network and pulmonary hypertension in CDH.

CELLULAR MODELS IN CDH RESEARCH

CDH cannot be attributed to one single source or defect, which makes it hard to study its pathogenesis, but new cell culture models can aid in improving insights at the cellular level. Recently, a cell culture model was described where PPFs from mice were isolated and cultured to outgrow and expand PPF fibroblasts, that maintained expression of key diaphragm genes (43). Pharmacological inhibition or genetic manipulation that causes CDH resulted in reduced *in vitro* proliferation in PPF-derived fibroblasts (43). Also, lung organoids were recently derived from induced pluripotent stem cells (iPSC) from fetuses and infants with CDH and showed reduced generation of lung progenitor cells and impaired epithelial- and mesenchymal differentiation (44). Recently, it was shown that organoid cultures can be obtained with a low input material from clinical samples, like tracheal aspirates from preterm newborns, and this method could also be used to grow organoids from CDH patients without the need of *in vitro* differentiation from iPSCs (45). Furthermore, endothelial cell culture models could aid in understanding defective endothelial cell function (40) and endothelial cell-SMC interaction (38). A differentiation protocol from human iPSCs to endothelial progenitor cells that mimics *in vivo* embryonic vascular development was recently published (42). This method can be used to generate iPSC-derived endothelial cells from CDH patients to eventually use these cells in cell co-culture systems, like organoid cultures. Although CDH is a multifactorial disease, *in vivo* experiments are limited and these kind of culture models will help to understand the pathology of CDH by specific cells, the interaction between different cell types and the molecular mechanisms in patient specific cultures.

DISCUSSION

In this review, we discussed cellular origins that are associated with CDH pathogenesis, including the diaphragm and PPF, lung mesothelium and (peri)vascular cells. All can contribute to a CDH phenotype, but also extrinsic factors play a role. Because pulmonary defects and alterations in the space of the chest cavity occurred prior to diaphragm closure and may even be the cause of a diaphragm defect (32), it is interesting to study the space in the chest cavity in other CDH models and potentially in human fetuses to serve as an early predictor for CDH. To mimic limited chest space and compression, lung organoids have been subjected to mechanical pressure, which altered their development (44). These results show that extrinsic factors play a significant role in CDH pathogenesis and the influence of limited space and compression should be studied further, since *Wt1* knock-out lungs still had the capacity to develop normally *ex vivo*, which is promising for potential treatment strategies (32).

Intrapleural delivery of compounds has been suggested to target PMCs in idiopathic pulmonary fibrosis, since this may result in increased efficacy combined with reduced systemic toxicity of therapeutic agents (19, 46). This delivery strategy may also be interesting for targeting PMCs during lung development. Mesothelial mobilization is not only implicated in development of the lungs, but also in other organs, like the liver, heart and reproductive system (47). However, it has been shown that this process can differ between different organs and that mesothelial migration in developing lungs differs in timing and pathway dependency compared to other organs, like the heart (48). These features could be used for organ-specific targeting and makes the embryonic mesothelium an interesting therapeutic target.

In summary, defective development, communication or migration of cells from the diaphragm, the mesothelium and (peri)vascular cells of the lungs play an important role in CDH pathogenesis. Improved *in vitro* cell culture models and the generation of patient-specific cultures will provide more insights in cellular and molecular mechanisms that underlie these defects and their use may be beneficial for identification and testing of putative therapeutic agents.

AUTHOR CONTRIBUTIONS

GE and GK wrote the manuscript. GS, RR, and DT carefully edited and revised the various versions of the manuscript and approved the final manuscript. RW revised and approved the final manuscript. All authors approved the final manuscript as submitted.

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REFERENCES

1. Guner YS, Delaplain PT, Zhang L, Di Nardo M, Brogan TV, Chen Y, et al. Trends in mortality and risk characteristics of congenital diaphragmatic hernia treated with extracorporeal membrane oxygenation. *ASAIO J.* (2019) 65:509–15. doi: 10.1097/MAT.0000000000000834
2. Gupta VS, Harting MT, Lally PA, Miller CC, Hirschl RB, Davis CF, et al. Has Survival improved for congenital diaphragmatic hernia? A 25-year review of over 5000 patients from the CDH study group. *Pediatrics.* (2021) 147(3 MeetingAbstract):939. doi: 10.1542/peds.147.3_MeetingAbstract.939
3. Schulz F, Jenetzky E, Zwink N, Bendixen C, Kipfmueller F, Rafat N, et al. Parental risk factors for congenital diaphragmatic hernia – a large German case-control study. *BMC Pediatrics.* (2021) 21:278. doi: 10.1186/s12887-021-02748-3
4. Mesas Burgos C, Ehrén H, Conner P, Frenckner B. Maternal risk factors and perinatal characteristics in congenital diaphragmatic hernia: a nationwide population-based study. *Fetal Diagnosis and Therapy.* (2019) 46:385–91. doi: 10.1159/000497619
5. Kardon G, Ackerman KG, McCulley DJ, Shen Y, Wynn J, Shang L, et al. Congenital diaphragmatic hernias: from genes to mechanisms to therapies. *Dis Models Mech.* (2017) 10:955–70. doi: 10.1242/dmm.028365
6. Merrell AJ, Kardon G. Development of the diaphragm – a skeletal muscle essential for mammalian respiration. *Febs J.* (2013) 280:4026–35. doi: 10.1111/febs.12274
7. Merrell AJ, Ellis BJ, Fox ZD, Lawson JA, Weiss JA, Kardon G. Muscle connective tissue controls development of the diaphragm and is a source of congenital diaphragmatic hernias. *Nat Genet.* (2015) 47:496–504. doi: 10.1038/ng.3250
8. Sefton EM, Gallardo M, Kardon G. Developmental origin and morphogenesis of the diaphragm, an essential mammalian muscle. *Dev Biol.* (2018) 440:64–73. doi: 10.1101/278507
9. Babiuk RP, Zhang W, Clugston R, Allan DW, Greer JJ. Embryological origins and development of the rat diaphragm. *J Comp Neurol.* (2003) 455:477–87. doi: 10.1002/cne.10503
10. Allan DW, Greer JJ. Embryogenesis of the phrenic nerve and diaphragm in the fetal rat. *J Comp Neurol.* (1997) 382:459–68. doi: 10.1002/(SICI)1096-9861(19970616)382:4<459::AID-CNE3>3.0.CO;2-1
11. Dietrich S, Abou-Rebyeh F, Brohmann H, Bladt F, Sonnenberg-Riethmacher E, Yamaai T, et al. The role of SF/HGF and c-Met in the development of skeletal muscle. *Development.* (1999) 126:1621–9. doi: 10.1242/dev.126.8.1621
12. Ackerman KG, Herron BJ, Vargas SO, Huang H, Tevosian SG, Kochilas L, et al. Fog2 is required for normal diaphragm and lung development in mice and humans. *PLoS Genet.* (2005) 1:58–65. doi: 10.1371/journal.pgen.0010010
13. Carmona R, Cañete A, Cano E, Ariza L, Muñoz-Chápuli R. Conditional deletion of WT1 in the septum transversum mesenchyme causes congenital diaphragmatic hernia in mice. *eLife.* (2016) 19:e16009. doi: 10.7554/eLife.16009.013
14. Clugston RD, Zhang W, Greer JJ. Gene expression in the developing diaphragm: significance for congenital diaphragmatic hernia. *Am J Physiol Lung Cell Mol Physiol.* (2008) 294:L665–75. doi: 10.1152/ajplung.00027.2008
15. Paris ND, Coles GL, Ackerman KG. Wt1 and beta-catenin cooperatively regulate diaphragm development in the mouse. *Dev Biol.* (2015) 407:40–56. doi: 10.1016/j.ydbio.2015.08.009
16. Clugston RD, Zhang W, Alvarez S, de Lera AR, Greer JJ. Understanding abnormal retinoid signaling as a causative mechanism in congenital diaphragmatic hernia. *Am J Respir Cell Mol Biol.* (2010) 42:276–85. doi: 10.1165/rcmb.2009-0076OC
17. Takayasu H, Nakazawa N, Montedonico S, Sugimoto K, Sato H, Puri P. Impaired alveolar epithelial cell differentiation in the hypoplastic lung in nitrofen-induced congenital diaphragmatic hernia. *Pediatr Surg Int.* (2007) 23:405–10. doi: 10.1007/s00383-006-1853-y
18. Nguyen TM, Jimenez J, Rendin LE, Müller C, Westergren-Thorsson G, Deprest J, et al. The proportion of alveolar type 1 cells decreases in murine hypoplastic congenital diaphragmatic hernia lungs. *PLoS ONE.* (2019) 14:e0214793. doi: 10.1371/journal.pone.0214793
19. Batra H, Antony VB. The pleura mesothelium in development and disease. *Front Physiol.* (2014) 5:284. doi: 10.3389/fphys.2014.00284
20. Mutsaers SE. Mesothelial cells: their structure, function and role in serosal repair. *Respirology.* (2002) 7:171–91. doi: 10.1046/j.1440-1843.2002.00404.x
21. Dixit R, Ai X, Fine A. Derivation of lung mesenchymal lineages from the fetal mesothelium requires hedgehog signaling for mesothelial cell entry. *Development.* (2013) 140:4398–406. doi: 10.1242/dev.098079
22. Wilm B, Muñoz-Chapuli R. The role of WT1 in embryonic development and normal organ homeostasis. In: Hastie N, editor. *The Wilms' Tumor (WT1) Gene: Methods and Protocols*. New York, NY: Springer New York (2016). p. 23–39.
23. Que J, Wilm B, Hasegawa H, Wang F, Bader D, Hogan BLM. Mesothelium contributes to vascular smooth muscle and mesenchyme during lung development. *Proc Natl Acad Sci USA.* (2008) 105:16626. doi: 10.1073/pnas.0808649105
24. Cano E, Carmona R, Muñoz-Chápuli R. Wt1-expressing progenitors contribute to multiple tissues in the developing lung. *Am J Physiol Lung Cell Mol Physiol.* (2013) 305:L322–32. doi: 10.1152/ajplung.00424.2012
25. von Gise A, Stevens SM, Honor LB, Oh JH, Gao C, Zhou B, et al. Contribution of Fetal, but not adult, pulmonary mesothelium to mesenchymal lineages in lung homeostasis and fibrosis. *Am J Respir Cell Mol Biol.* (2016) 54:222–30. doi: 10.1165/rcmb.2014-0461OC

26. Danopoulos S, Shiosaki J, Al Alam D. FGF signaling in lung development and disease: human versus mouse. *Front Genet.* (2019) 10:170. doi: 10.3389/fgene.2019.00170
27. White AC, Xu J, Yin Y, Smith C, Schmid G, Ornitz DM. FGF9 and SHH signaling coordinate lung growth and development through regulation of distinct mesenchymal domains. *Development.* (2006) 133:1507–17. doi: 10.1242/dev.02313
28. Yin Y, Ornitz DM. FGF9 and FGF10 activate distinct signaling pathways to direct lung epithelial specification and branching. *Sci Signal.* (2020) 13(621). doi: 10.1126/scisignal.aay4353
29. Yin Y, Wang F, Ornitz DM. Mesothelial- and epithelial-derived FGF9 have distinct functions in the regulation of lung development. *Development.* (2011) 138:3169–77. doi: 10.1242/dev.065110
30. Takahashi H, Friedmacher F, Fujiwara N, Hofmann A, Puri P. Pulmonary FGF9 gene expression is downregulated during the pseudoglandular stage in nitrofen-induced hypoplastic lungs. *Eur J Pediatr Surg.* (2014) 24:75–8. doi: 10.1055/s-0033-1351392
31. van Loenhout RB, Tseu I, Fox EK, Huang Z, Tibboel D, Post M, et al. The pulmonary mesenchymal tissue layer is defective in an *in vitro* recombinant model of nitrofen-induced lung hypoplasia. *Am J Pathol.* (2012) 180:48–60. doi: 10.1016/j.ajpath.2011.09.032
32. Gilbert RM, Schappell LE, Gleghorn JP. Defective mesothelium and limited physical space are drivers of dysregulated lung development in a genetic model of congenital diaphragmatic hernia. *Development.* (2021) 148:dev199460. doi: 10.1242/dev.199460
33. Kool H, Mous D, Tibboel D, de Klein A, Rottier RJ. Pulmonary vascular development goes awry in congenital lung abnormalities. *Birth Defects Res C Embryo Today Rev.* (2014) 102:343–58. doi: 10.1002/bdrc.21085
34. Varghese NP, Tillman RH, Keller RL. Pulmonary hypertension is an important co-morbidity in developmental lung diseases of infancy: bronchopulmonary dysplasia and congenital diaphragmatic hernia. *Pediatr Pulmonol.* (2021) 56:670–7. doi: 10.1002/ppul.25258
35. Mous DS, Kool HM, Wijnen R, Tibboel D, Rottier RJ. Pulmonary vascular development in congenital diaphragmatic hernia. *Eur Respir Rev.* (2018) 27:170104. doi: 10.1183/16000617.0104-2017
36. Sluiter I, van der Horst I, van der Voorn P, Boerema-de Munck A, Buscop-van Kempen M, de Krijger R, et al. Premature differentiation of vascular smooth muscle cells in human congenital diaphragmatic hernia. *Exp Mol Pathol.* (2013) 94:195–202. doi: 10.1016/j.yexmp.2012.09.010
37. Yang MJ, Russell KW, Yoder BA, Fenton SJ. Congenital diaphragmatic hernia: a narrative review of controversies in neonatal management. *Transl Pediatr.* (2021) 10:1432–47. doi: 10.21037/tp-20-142
38. Acker SN, Seedorf GJ, Abman SH, Nozik-Grayck E, Kuhn K, Partrick DA, et al. Altered pulmonary artery endothelial–smooth muscle cell interactions in experimental congenital diaphragmatic hernia. *Pediatr Res.* (2015) 77:511–9. doi: 10.1038/pr.2015.13
39. Kool HM, Bürgisser PE, Edel GG, de Kleer I, Boerema-de Munck A, de Laat I, et al. Inhibition of retinoic acid signaling induces aberrant pericyte coverage and differentiation resulting in vascular defects in congenital diaphragmatic hernia. *Am J Physiol Lung Cell Mol Physiol.* (2019) 317:L317–L31. doi: 10.1152/ajplung.00104.2018
40. Acker SN, Seedorf GJ, Abman SH, Nozik-Grayck E, Partrick DA, Gien J. Pulmonary artery endothelial cell dysfunction and decreased populations of highly proliferative endothelial cells in experimental congenital diaphragmatic hernia. *Am J Physiol Lung Cell Mol Physiol.* (2013) 305:L943–L52. doi: 10.1152/ajplung.00226.2013
41. Wakabayashi T, Naito H, Suehiro JI, Lin Y, Kawaji H, Iba T, et al. CD157 marks tissue-resident endothelial stem cells with homeostatic and regenerative properties. *Cell Stem Cell.* (2018) 22:384–97 e6. doi: 10.1016/j.stem.2018.01.010
42. Farkas S, Simara P, Rehakova D, Veverkova L, Koutna I. Endothelial progenitor cells produced from human pluripotent stem cells by a synergistic combination of cytokines, small compounds, and serum-free medium. *Front Cell Dev Biol.* (2020) 8:309. doi: 10.3389/fcell.2020.00309
43. Bogenschutz EL, Sefton EM, Kardon G. Cell culture system to assay candidate genes and molecular pathways implicated in congenital diaphragmatic hernias. *Dev Biol.* (2020) 467:30–8. doi: 10.1016/j.ydbio.2020.07.013
44. Kunisaki SM, Jiang G, Biancotti JC, Ho KKY, Dye BR, Liu AP, et al. Human induced pluripotent stem cell-derived lung organoids in an ex vivo model of the congenital diaphragmatic hernia fetal lung. *STEM Cells Transl Med.* (2021) 10:98–114. doi: 10.1002/sctm.20-0199
45. Eenjes E, van Riet S, Kroon AA, Slats AM, Khedoe PPSJ, Boerema-de Munck A, et al. Disease modelling following organoid-based expansion of airway epithelial cells. *Am J Physiol Lung Cell Mol Physiol.* (2021) 321:L775–86. doi: 10.1152/ajplung.00234.2020
46. Hocking A, Tommasi S, Sordillo P, Klebe S. The safety and exploration of the pharmacokinetics of intrapleural liposomal curcumin. *Int J Nanomedicine.* (2020) 15:943–52. doi: 10.2147/IJN.S237536
47. Koopmans T, Rinkevich Y. Mesothelial to mesenchyme transition as a major developmental and pathological player in trunk organs and their cavities. *Commun Biol.* (2018) 1:170. doi: 10.1038/s42003-018-0180-x
48. Timo HL, Carsten R, Jennifer K, Regine H, Franziska G, Irina W, et al. Mesothelial mobilization in the developing lung and heart differs in timing, quantity, and pathway dependency. *Am J Physiol Lung Cell Mol Physiol.* (2019) 316:L767–83. doi: 10.1152/ajplung.00212.2018

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REVIEW
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Knowledge Gaps in the Fetal to Neonatal Transition of Infants With a Congenital Diaphragmatic Hernia

Philip L. J. DeKoninck[1,2*], *Emily J. J. Horn-Oudshoorn*[3], *Ronny Knol*[3], *Kelly J. Crossley*[2,4] *and Irwin K. M. Reiss*[3]

[1] Department of Obstetrics and Gynecology, Division Fetal Medicine, Erasmus MC University Medical Center—Sophia Children's Hospital, Rotterdam, Netherlands, [2] The Ritchie Centre, Hudson Institute of Medical Research, Melbourne, VIC, Australia, [3] Department of Pediatrics, Division of Neonatology, Erasmus MC University Medical Center—Sophia Children's Hospital, Rotterdam, Netherlands, [4] Department of Obstetrics and Gynaecology, School of Clinical Sciences, Monash University, Melbourne, VIC, Australia

Clinical research for infants born with a congenital diaphragmatic hernia (CDH) has until recently mainly focused on advances in prenatal and postnatal treatment. However, during the early perinatal transition period there are major physiological adaptations. For most infants these changes will happen uneventfully, but for CDH infants this marks the beginning of serious respiratory complications. In recent years, there is emerging evidence that the clinical management during the perinatal stabilization period in the delivery room may influence postnatal outcomes. Herein, we discuss major knowledge gaps and novel concepts that aim to optimize fetal to neonatal transition for infants with CDH. One such novel and interesting approach is performing resuscitation with an intact umbilical cord, the efficacy of this procedure is currently being investigated in several clinical trials. Furthermore, close evaluation of neonatal physiological parameters in the first 24 h of life might provide early clues concerning the severity of lung hypoplasia and the risk of adverse outcomes. We will provide an overview of trending concepts and discuss potential areas for future research.

Keywords: congenital diaphragmatic hernia, birth, cord clamping, neonatal transition, oxygen, respiratory monitoring

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***Correspondence:**
Philip L. J. DeKoninck
p.dekoninck@erasmusmc.nl

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INTRODUCTION

The management of infants with a congenital diaphragmatic hernia (CDH) is continuously evolving with major improvements in prenatal and postnatal care. Most advances are based on solid scientific evidence using available animal models of CDH prior to translating it into the clinical setting (1). For many of the *in vivo* experiments done in small animals (rabbit, rat and mice models), the endpoint is birth given the lethality of the condition without intensive care. To investigate novel concepts in early postnatal care large animal models (such as the ovine model) are often necessary, yet these experiments are costly and require a dedicated research facility.

Until recently, the transition period defined as the time immediately after birth, has been relatively overlooked. In fact, for a long time the main intervention was to clamp the cord and transfer the infant to the resuscitation table for further stabilization as soon as possible (2–4). On the other hand, major physiological adaptations occur during this immediate postnatal period and a complicated course may effect long term outcomes (5).

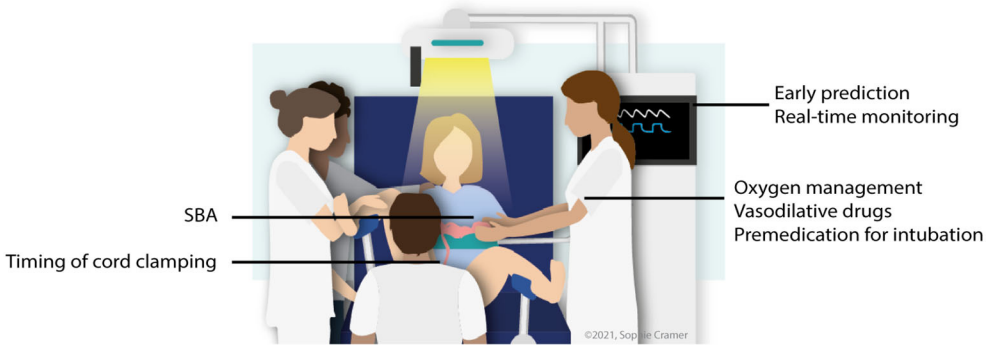

FIGURE 1 | Overview of potential research areas concerning perinatal stabilization of CDH infants. SBA, spontaneous breathing approach. Adapted from Knol et al., (15) with permission of the illustrator.

In the past decades there has been tremendous effort invested in optimizing the perinatal stabilization period for infants born preterm with immature lungs or those that may undergo problematic fetal to neonatal transition; such as due to birth asphyxia or in case of an elective cesarean section (6, 7). Our knowledge of the physiology underpinning the changes at birth has dramatically improved and novel concepts concerning the timing of cord clamping, oxygen management and the type and level of respiratory support required were introduced to clinical practice (7, 8).

Some of these approaches are now being evaluated in large clinical trials, but the promising preliminary results have also inspired researchers to investigate their effectiveness for conditions that affect *in-utero* lung development, such as CDH (9–14). Research about neonatal transition for infants with a CDH is rapidly developing, in this literature review we describe new insights and we discuss knowledge gaps for future research (**Figure 1**).

INTACT CORD RESUSCITATION

For most infants, adequate gas-exchange is promptly established after birth, i.e., within the first breaths, by rapid clearance of lung liquid resulting in aeration of the lungs. However, infants born with lung hypoplasia have a reduced liquid clearance rate, which is proportional to the lung size and thus reduces the infant's ability to aerate its lungs (16). This problem is likely a reflection of a simplified distal airway architecture and as such a reduced cross sectional area for moving lung liquid into the interstitial tissues. Furthermore, hypoplastic lungs generally have a higher elastic recoil (stiffer) demonstrated by a lower dynamic lung compliance (10, 16, 17).

Lung aeration is considered a key factor in driving vasodilation of the pulmonary vasculature and thereby increasing pulmonary blood flow (18). Apart from establishing adequate gas-exchange, lung aeration is essential for a smooth cardiovascular transition from a fetus to a newborn. Immediately after umbilical cord clamping there is a sudden increase in peripheral vascular resistance and at the same instant venous return to the left atrium via the ductus venosus and foramen ovale stops. In the hypoplastic lung, pulmonary vascular resistance remains high and therefore adequate left venous return is not rapidly restored, whereas in normally developed lungs venous return is established within the first breaths (10, 18). A delayed restoration of venous return translates in a sudden decrease in cardiac output (30–50% reduction) and neonatal hypoxemia, which is considered a risk factor for developing persistent pulmonary hypertension (9). Furthermore, the impaired vascular relaxation forces higher pulmonary perfusion pressures to maintain adequate pulmonary blood flow. We have recently shown that in a lamb CDH model, after an initial improvement in pulmonary vascular resistance, this short period of exposure to higher driving pressures may be a trigger for developing pulmonary hypertension at a later stage (9). This observation could be the physiological explanation of the so-called 'honeymoon' period, a transient time of clinical stability, that is observed in some infants with CDH (19).

In recent years, the importance of delaying cord clamping until after lung aeration (and adequate left venous return) has gained momentum, specifically in preterm infants born with immature lungs. Likewise, there have recently been two feasibility studies evaluating this approach for infants born with CDH (11, 12). An important consideration is the need to provide mechanical ventilation to the neonate in close proximity to the mother whilst the integrity of the umbilical cord remains intact. A mobile resuscitation trolley is required for this approach to be successful and several alternatives are currently commercially available (20). These trolleys have inherent limitations and advantages, which are important to consider when implementing intact cord resuscitation, as well as the financial aspect given considerable differences in acquisition costs.

Both studies, although small sample sizes ($n = 20$), reported good feasibility of 85% and 100%, respectively (11, 12). It is obviously not possible to draw firm conclusions, however both found improved cardiovascular adaptation, resulting in higher blood pressures, less need for cardiac resuscitation and higher Apgar scores (11, 12). These promising findings led to the initiation of two large randomized trials: Congenital Hernia

Intact Cord (CHIC, *NCT04429750*) and Physiological-based cord clamping for infants with a Congenital Diaphragmatic Hernia (PinC, *NCT04373902*) (21).

These two trials aim to defer cord clamping until after the infant's lungs are aerated, which is challenging to determine. CO_2 detectors, respiratory monitors or bedside echocardiography (ductus arteriosus evaluation) could be used for this purpose, but they have inherent technical limitations or are logistically not always feasible in the immediate postnatal setting. Hence, physiological parameters such as heart rate, oxygen saturation and the level of oxygen supplementation are considered as a good alternate proxies for determining lung aeration and the state of the infant's cardiovascular adaptation (9, 21). In the future, with the rapid improvement of bedside respiratory monitors, real-time evaluation of tidal volumes or other lung mechanics might be another way to ascertain adequate lung aeration.

The other challenge is to define a clinically relevant primary outcome. The ultimate endpoint is survival to discharge, however despite efforts to standardize postnatal management, considerable bias due to variations in local management make it difficult to determine the actual benefit of performing intact cord resuscitation. The concern of bias is even more pronounced in multicenter trials, however given the incidence of CDH and the required sample sizes it is almost impossible to investigate this in a single center setting. Consequently, short term outcomes such as Apgar scores (CHIC) and pulmonary hypertension (PinC) were chosen as alternative primary outcomes.

The results of these clinical trials are expected in the next two to three years. Despite differences in methodology, a subsequent meta-analysis using individual participant data might strengthen the scientific evidence physiologically based cord clamping even further.

SPONTANEOUS BREATHING APPROACH

For most infants with CDH, mechanical ventilation is a double-edged sword: it is essential for survival, but prolonged respiratory support also poses a risk of iatrogenic complications such as ventilator-induced lung injury. On the other hand, a small subset of infants born with a very small diaphragmatic defect, hence mild lung hypoplasia, may not develop severe respiratory insufficiency immediately after birth and thus mechanical ventilation may not be necessary. In fact, prior to the routine use of ultrasound this was probably the group that survived the neonatal period with minimal care and that was only diagnosed at childhood age. Moreover, prompt intubation after birth potentially causes stress and pain for the infant, thereby triggering the development of pulmonary hypertension and impacting neonatal transition. Regardless, the main purpose of routine intubation at birth is to avoid transient hypoxia, which is considered an even more important trigger for pulmonary hypertension (22).

We have recently published our experience of adopting a gentler approach during the initial perinatal stabilization phase by allowing spontaneous breathing in a select subset of patients (23). This approach was only offered for infants with an isolated left-sided CDH, born >35 weeks' gestation and predicted to have a very mild degree of lung hypoplasia. The latter was determined by an observed/expected lung-to-head ratio above 50% and an intra-abdominal position of the liver. In this small study ($n = 15\%$), 40% of cases were only intubated at the time of postnatal correction of the diaphragmatic defect, thus required limited respiratory support and were discharged earlier from the intensive care unit. But more importantly, a trial of spontaneous breathing, even unsuccessful, did not appear to impact survival or short-term morbidity (23).

In attempt to improve the success rate of this SBA we have drafted a consensus protocol after several meetings with international experts on CDH management, neonatal resuscitation and fetal/neonatal physiology. One of the recommendations is to start non-invasive respiratory support in these infants, as many of the infants in the above-mentioned series required low flow oxygen supplementation or continuous positive airway pressure (CPAP). However, it is not clear whether the key component to facilitate neonatal transition is either oxygen supplementation, distending airway pressure or a combination of both. The use of positive pressure ventilation is certainly controversial as the main concern is insufflation of the digestive tract, which may impair and limit lung expansion. Therefore, nasal high flow therapy could be an interesting option, because it is relatively easy to position the device, well-tolerated and provides a vehicle for oxygen delivery but also generates distending airway pressure. The level of respiratory support can be adjusted based on the individual needs by changing the flow rate and/or concentration of oxygen delivered. An alternative could be the use of a CPAP mask rather than nasal prongs because it is easier to position. Regardless of the treatment modality used, early insertion of a naso- or orogastric tube with continuous section is advised to avoid stomach distention. The result of these consensus meetings is a proposed algorithm for SBA comparable to what is used in the current neonatal resuscitation guidelines, and this protocol will be published soon.

There is an increasing number of centers that are considering or already have started attempting a trial of spontaneous breathing for infants with mild lung hypoplasia. In the absence of a randomized trial, it is essential to collect the outcome data and given the rareness of the abnormality, a multicenter and international collaboration is the most logical step. To accommodate this, a research consortium was founded consisting of partners all over Europe and Australia: Very mild CDH–Spontaneous Breathing Approach; VeSBA. Outcome data will be collected prospectively in a web-based registry.

SEDATE OR NOT SEDATE?

The majority of infants with a CDH will be intubated immediately after birth. Given the urgency to commence respiratory support in these infants and usually the lack of intravenous access intubation is often done without administering any sedation. The physiological responses to awake intubation of neonates are well-described (24, 25). It can be painful for the infant, translating into markers of acute stress such as increased intracranial and systemic blood pressure, bradycardia and reduced transcutaneous oxygen

saturation (24, 25). Furthermore, mediastinal shift and neonatal movements can complicate intubation resulting in a higher stress level for the infant. Therefore, in (semi) elective intubation premedication is considered good standard of care. For CDH infants, the priority is on establishing a secure airway for mechanical ventilation and thus vascular access is often obtained later in the stabilization phase. Alternative options to administer drugs are via the umbilical vein (direct puncture, not via a catheter), buccal or intranasal, however the interval to the onset of effect is potentially longer with the latter two (26, 27). In addition, there is an important knowledge gap when it comes to the optimal treatment regimen (type, dosage) of the premedication (28).

OXYGEN MANAGEMENT

Oxygen supplementation is an essential part of perinatal stabilization of an infant with CDH. The aim is to avoid arterial hypoxemia as it may trigger a vasoactive response and many clinicians will initiate oxygen supplementation with 100% oxygen. After the initial stabilization in the delivery room, oxygen administration is titrated based on the infant's needs targeting a pre-ductal saturation of between 80 and 95% (2). In any case, hyperoxia should be avoided because it also has adverse effects by producing oxygen free radicals. This consideration is certainly important for infants with a relative mild degree of lung hypoplasia, as using 100% oxygen supplementation might be counter effective. Oxidative stress and oxygen free radicals are not only associated with short-term neonatal morbidity but may have long lasting influence on development (29, 30). It has been recently demonstrated that even a brief period of high oxygen exposure may attenuate vasoactive response of the pulmonary vessels to treatments such as inhaled nitric oxide (31).

An alternative approach would be to start stabilization with a reduced oxygen concentration and a stepwise increase or decrease guided by the infant's saturation values (13). This approach is comparable to the resuscitation guidelines for preterm infants (8). In a recent series, the safety of such an alternative approach was evaluated, observing comparable rates of perinatal survival, ECMO use and duration of mechanical ventilation compared to historical CDH controls (13). Moreover, the need of 100% oxygen during the perinatal stabilization period provided an early indication of disease severity and subsequent adverse outcomes (13).

Another important knowledge gap is the use of supplemental oxygen during resuscitation of CDH infants whilst the umbilical cord is still intact. A recent study showed that in preterm lambs, pulmonary blood flow was considerably higher to controls when using 100% oxygen during delayed cord clamping (32). Interestingly, this was not causing systemic hyperoxygenation and hypothetically, the placenta may act as a buffer to reduce the arterial oxygen saturation (32).

The degree of supplemental oxygen exposure at birth may also be diminished by initiating vasodilative treatments already during neonatal resuscitation, such as inhaled nitric oxide, as was recently observed in a small series of preterm infants (33). We speculate that using such an approach for CDH infants may facilitate decreasing pulmonary vascular resistance after birth, thereby preventing high perfusion pressures and potentially avoiding a dysregulated vascular tone of the lung vessels (34). Hypothetically, combining this approach with deferring cord clamping until the lungs are aerated, both appear to have a protective effect on the lung vessels, may have a synergistic effect (9).

EARLY PREDICTORS OF ADVERSE OUTCOME

Infants born with CDH will only face respiratory challenges after birth and consequently it is only at that moment that clinicians can determine the true impact on lung development. Prenatal ultrasound and fetal MRI have proven to be very useful in the individual prediction of prognosis, yet it remains challenging to perform a functional assessment of the lungs (35). More specifically, the occurrence and severity of pulmonary hypertension or cardiac dysfunction are difficult to predict given the differences between the fetal and the neonatal circulation.

The immediate postpartum period provides clinicians a first glance of the infant's respiratory capacity. Consequently, this period also enables clinicians a chance to determine the severity of the congenital abnormality by monitoring physiological parameters and/or ventilatory requirements. There are already several scoring systems to determine the risk of adverse outcomes available, such as the Score for Neonatal Acute Physiology–II (SNAP-II) score, Wilford Hall/Santa Rosa prediction model and the Brindle scoring model (36–40). Most of these scoring systems are combining several clinical parameters (such as blood pressure, serum pH, fraction of inspired oxygen FiO_2) yet the majority of these scoring systems use the worst values of the parameters within the first 12–24 h (37–39).

We speculate that the level of respiratory support required during the initial neonatal resuscitation may provide important information of possible adverse outcomes. For instance, as described above, the need to supplement with a high FiO_2 concentration in the delivery room appears to be associated with higher morbidity and mortality for CDH infants (13, 41). Likewise, similar observations were reported regarding expiratory tidal volumes, end-tidal carbon dioxide levels and dynamic lung compliance (41, 42). Respiratory monitors now allow real-time measurements of several lung function parameters. Recording these parameters gives an opportunity to gather large datasets by aggregating individual patient data within a framework of multicenter collaborations, which can be used for prediction modeling to identify early signs of deterioration. In addition, combining physiological and ventilatory outcome measures, such as is done with the oxygen saturation index (OSI), may improve

the predictive value of these models for adverse models even further (36).

CONCLUSION

Herein, we have described some of the trending new concepts regarding interventions in the early perinatal stabilization phase for infants with CDH. Our understanding of the physiological adaptations immediately after birth has certainly grown in recent decades, but considerable knowledge gaps are remaining. Regardless, thorough investigation using appropriate preclinical models is essential prior to translating novel concepts into clinical practice. Importantly, optimizing the fetal to neonatal transition will not only improve postnatal outcomes for infants born with CDH, but also for those born with abnormal lung development caused by a broad range of other conditions, such as prolonged anhydramnios.

AUTHOR CONTRIBUTIONS

PD and IR were involved in the conception of this paper and wrote the first draft, which was critically reviewed by all authors. The final version was approved by all authors.

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REFERENCES

1. van Loenhout RB, Tibboel D, Post M, Keijzer R. Congenital diaphragmatic hernia: comparison of animal models and relevance to the human situation. *Neonatology.* (2009) 96:137–49. doi: 10.1159/000209850
2. Snoek KG, Reiss IKMM, Greenough A, Capolupo I, Urlesberger B, Wessel L, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH EURO Consortium Consensus−2015 Update. *Neonatology.* (2016) 110:66–74. doi: 10.1159/000444210
3. Puligandla PS, Skarsgard ED, Offringa M, Adatia I, Baird R, Bailey JAM, et al. Diagnosis and management of congenital diaphragmatic hernia: a clinical practice guideline. *CMAJ.* (2018) 190:E103–12. doi: 10.1503/cmaj.170206
4. Jancelewicz T, Brindle ME, Guner YS, Lally PA, Lally KP, Harting MT. Toward standardized management of congenital diaphragmatic hernia: an analysis of practice guidelines. *J Surg Res.* (2019) 243:229–35. doi: 10.1016/j.jss.2019.05.007
5. Horn-Oudshoorn EJJ, Knol R, Te Pas AB, Hooper SB, Cochius-Den Otter SCM, Wijnen RMH, et al. Perinatal stabilisation of infants born with congenital diaphragmatic hernia: a review of current concepts. *Arch Dis Child Fetal Neonatal Ed.* (2020) 105:449–54. doi: 10.1136/archdischild-2019-318606
6. Stuart Hooper BB, Binder-Heschl C, Polglase R, Gill W, Kluckow M, Wallace EM, et al. Maternal health, neonatology and perinatology the timing of umbilical cord clamping at birth: physiological considerations. *Matern Heal Neonatol Perinatol.* (2016) 2:1–9. doi: 10.1186/s40748-016-0032-y
7. te Pas A, Roehr CC, Foglia EE, Hooper S. Neonatal resuscitation research: closing the gap. *Pediatr Res.* (2021) 1–3. doi: 10.1038/s41390-021-01403-y
8. Wyckoff MH, Wyllie J, Aziz K, de Almeida MF, Fabres JW, Fawke J, et al. Neonatal life support 2020 international consensus on cardiopulmonary resuscitation and emergency cardiovascular care science with treatment recommendations. *Resuscitation.* (2020) 156:A156–87. doi: 10.1016/j.resuscitation.2020.09.015
9. Kashyap AJ, Hodges RJ, Thio M, Rodgers KA, Amberg BJ, McGillick E V., et al. Physiologically based cord clamping improves cardiopulmonary haemodynamics in lambs with a diaphragmatic hernia. *Arch Dis Child Fetal Neonatal Ed.* (2019) 105:18–25. doi: 10.1016/j.ajog.2018.11.1016
10. Kashyap AJ, Crossley KJ, DeKoninck PLJ, Rodgers KA, Thio M, Skinner SM, et al. Neonatal cardiopulmonary transition in an ovine model of congenital diaphragmatic hernia. *Arch Dis Child Fetal Neonatal Ed.* (2019) 104:F617–23. doi: 10.1136/archdischild-2018-316045
11. Lefebvre C, Rakza T, Weslinck N, Vaast P. Houfflin-debarge V, Mur S, Storme L. Feasibility and safety of intact cord resuscitation in newborn infants with congenital diaphragmatic hernia (CDH). *Resuscitation.* (2017) 120:20–5. doi: 10.1016/j.resuscitation.2017.08.233
12. Foglia EE, Ades A, Hedrick HL, Rintoul N, Munson DA, Moldenhauer J, et al. Initiating resuscitation before umbilical cord clamping in infants with congenital diaphragmatic hernia: a pilot feasibility trial. *Arch Dis Child Fetal Neonatal Ed.* (2019) 105:322–6. doi: 10.1136/archdischild-2019-317477
13. Riley JS, Antiel RM, Rintoul NE, Ades AM, Waqar LN, Lin N, et al. Reduced oxygen concentration for the resuscitation of infants with congenital diaphragmatic hernia. *J Perinatol.* (2018) 38:834–43. doi: 10.1038/s41372-017-0031-5
14. Snoek KG, Capolupo I, Van Rosmalen J, De Jongste-Van Den Hout L, Vijfhuize S, Greenough A, et al. Conventional mechanical ventilation versus high-frequency oscillatory ventilation for congenital diaphragmatic hernia. A randomized clinical trial (The VICI-trial). *Ann Surg.* (2016) 263:867–74. doi: 10.1097/SLA.0000000000001533
15. Knol R, Brouwer E, Akker TV, DeKoninck P, Geloven NV, Polglase GR, et al. Physiological-based cord clamping in very preterm infants - randomised controlled trial on effectiveness of stabilisation. *Resuscitation.* (2020) 147:26–33. doi: 10.1016/j.resuscitation.2019.12.007
16. Flemmer AW, Thio M, Wallace MJ, Lee K, Kitchen MJ, Kerr L, et al. Lung hypoplasia in newborn rabbits with a diaphragmatic hernia affects pulmonary ventilation but not perfusion. *Pediatr Res.* (2017) 82:536–43. doi: 10.1038/pr.2017.91
17. McGillick E V, Davies IM, Hooper SB, Kerr LT, Thio M, DeKoninck P, et al. Effect of lung hypoplasia on the cardiorespiratory transition in newborn lambs. *J Appl Physiol.* (2019) 127:568–78. doi: 10.1152/japplphysiol.00760.2018
18. Hooper SB, Te Pas AB, Lang J, Van Vonderen JJ, Roehr CC, Kluckow M, et al. Cardiovascular transition at birth: a physiological sequence. *Pediatr Res.* (2015) 77:608–14. doi: 10.1038/pr.2015.21
19. Geggel RL, Murphy JD, Langleben D, Crone RK, Vacanti JP, Reid LM. Congenital diaphragmatic hernia: arterial structural changes and persistent pulmonary hypertension after surgical repair. *J Pediatr.* (1985) 107:457–64. doi: 10.1016/S0022-3476(85)80534-5
20. Katheria A, Lee HC, Knol R, Irvine L, Thomas S, A. review of different resuscitation platforms during delayed cord clamping. *J Perinatol.* (2021) 41:1540–8. doi: 10.1038/s41372-021-01052-3
21. Duc L, Mur S, Rakza T, Boukhris MR, Rousset C, Vaast P, et al. Efficacy of intact cord resuscitation compared to immediate cord clamping on cardiorespiratory adaptation at birth in infants with isolated congenital diaphragmatic hernia (CHIC). *Children (Basel).* (2021) 8:339. doi: 10.3390/children8050339
22. Storme L, Aubry E, Rakza T, Houeijeh A, Debarge V, Tourneux P, et al. Pathophysiology of persistent pulmonary hypertension of the newborn: impact of the perinatal environment. *Arch Cardiovasc Dis.* (2013) 106:169–77. doi: 10.1016/j.acvd.2012.12.005

23. Cochius-den Otter SCM, Horn-Oudshoorn EJJ, Allegaert K, DeKoninck PLJ, Peters NCJ, Cohen-Overbeek TE, et al. Routine intubation in newborns with congenital diaphragmatic hernia. *Pediatrics.* (2020) 146:e20201258. doi: 10.1542/peds.2020-1258
24. Carbajal R, Eble B, Anand KJS. Premedication for tracheal intubation in neonates: confusion or controversy? *Semin Perinatol.* (2007) 31:309–17. doi: 10.1053/j.semperi.2007.07.006
25. Caldwell CD, Watterberg KL. Effect of premedication regimen on infant pain and stress response to endotracheal intubation. *J Perinatol.* (2015) 35:415–8. doi: 10.1038/jp.2014.227
26. McPherson C, Ortinau CM, Vesoulis Z. Practical approaches to sedation and analgesia in the newborn. *J Perinatol.* (2021) 41:383–95. doi: 10.1038/s41372-020-00878-7
27. Borrhomée S, Merbouche S, Kern-Duciau N, Boize P. Umbilical vein catheterization through Wharton's jelly: a possibility for a fast and safe way to deliver treatments in the delivery room? *Arch Pediatr.* (2019) 26:381–4. doi: 10.1016/j.arcped.2019.05.004
28. Shay R, Weikel BW, Grover T, Barry JS. Standardizing premedication for non-emergent neonatal tracheal intubations improves compliance and patient outcomes. *J Perinatol.* (2021) 1–7. doi: 10.1038/s41372-021-01215-2
29. Andresen JH, Saugstad OD. Oxygen metabolism and oxygenation of the newborn. *Semin Fetal Neonatal Med.* (2020) 25:101078. doi: 10.1016/j.siny.2020.101078
30. Perrone S, Bracciali C, Di Virgilio N, Buonocore G. Oxygen use in neonatal care: a two-edged sword. *Front Pediatr.* (2017) 4:143. doi: 10.3389/fped.2016.00143
31. Lakshminrusimha S, Russell JA, Steinhorn RH, Swartz DD, Ryan RM, Gugino SF, et al. Pulmonary hemodynamics in neonatal lambs resuscitated with 21%, 50%, and 100% oxygen. *Pediatr Res.* (2007) 62:313–8. doi: 10.1203/PDR.0b013e3180db29fe
32. Lakshminrusimha S, Vali P, Chandrasekharan P, Rich W, Katheria A. Differential alveolar and systemic oxygenation during preterm resuscitation with 100% oxygen during delayed cord clamping. *Am J Perinatol.* (2021) doi: 10.1055/s-0041-1730362
33. Sekar K, Szyld E, McCoy M, Wlodaver A, Dannaway D, Helmbrecht A, et al. Inhaled nitric oxide as an adjunct to neonatal resuscitation in premature infants: a pilot, double blind, randomized controlled trial. *Pediatr Res.* (2020) 87:523–8. doi: 10.1038/s41390-019-0643-x
34. Mous DS, Kool HM, Wijnen R, Tibboel D, Rottier RJ. Pulmonary vascular development in congenital diaphragmatic hernia. *Eur Respir Rev.* (2018) 27:1–6. doi: 10.1183/16000617.0104-2017
35. Cordier AG, Russo FM, Deprest J, Benachi A. Prenatal diagnosis, imaging, and prognosis in Congenital Diaphragmatic Hernia. *Semin Perinatol.* (2020) 44:51163. doi: 10.1053/j.semperi.2019.07.002
36. Rawat M, Chandrasekharan PK, Williams A, Gugino S, Koenigsknecht C, Swartz D, et al. Oxygen saturation index and severity of hypoxic respiratory failure. *Neonatology.* (2015) 107:161–6. doi: 10.1159/000369774
37. Snoek KG, Capolupo I, Morini F, Van Rosmalen J, Greenough A, Van Heijst A, et al. Score for neonatal acute physiology-II predicts outcome in congenital diaphragmatic hernia patients∗. *Pediatr Crit Care Med.* (2016) 17:540–6. doi: 10.1097/PCC.0000000000000738
38. Schultz CM, DiGeronimo RJ, Yoder BA. Congenital diaphragmatic hernia: a simplified postnatal predictor of outcome. *J Pediatr Surg.* (2007) 42:510–6. doi: 10.1016/j.jpedsurg.2006.10.043
39. Brindle ME, Cook EF, Tibboel D, Lally PA, Lally KP. A clinical prediction rule for the severity of congenital diaphragmatic hernias in newborns. *Pediatrics.* (2014) 134:e413–9. doi: 10.1542/peds.2013-3367
40. Cochius-Den Otter SCM, Erdem Ö, van Rosmalen J, Schaible T, Peters NCJ, Cohen-Overbeek TE, et al. Validation of a prediction rule for mortality in congenital diaphragmatic hernia. *Pediatrics.* (2020) 145:e20192379. doi: 10.1542/peds.2019-2379
41. O'Rourke-Potocki A, Ali K, Murthy V, Milner A, Greenough A. Resuscitation of infants with congenital diaphragmatic hernia. *Arch Dis Child Fetal Neonatal Ed.* (2017) 102:F320–3. doi: 10.1136/archdischild-2016-311432
42. Mank A, Carrasco Carrasco C, Thio M, Clotet J, Pauws SC, DeKoninck P, et al. Tidal volumes at birth as predictor for adverse outcome in congenital diaphragmatic hernia. *Arch Dis Child Fetal Neonatal Ed.* (2019) 105:248–52. doi: 10.1136/archdischild-2018-316504

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ORIGINAL RESEARCH
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Longitudinal Follow-Up With Radiologic Screening for Recurrence and Secondary Hiatal Hernia in Neonates With Open Repair of Congenital Diaphragmatic Hernia—A Large Prospective, Observational Cohort Study at One Referral Center

Katrin B. Zahn[1,2*], *Thomas Schaible*[2,3], *Neysan Rafat*[3], *Meike Weis*[4], *Christel Weiss*[5] *and Lucas Wessel*[1,2]

[1] Department of Pediatric Surgery, University Children's Hospital Mannheim, University of Heidelberg, Mannheim, Germany, [2] ERNICA-Center, Mannheim, Germany, [3] Department of Neonatology, University Children's Hospital Mannheim, University of Heidelberg, Mannheim, Germany, [4] Department of Clinical Radiology and Nuclear Medicine, University Medical Center Mannheim, University of Heidelberg, Mannheim, Germany, [5] Department of Medical Statistics and Biomathematics, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany

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University Hospitals Leuven, Belgium
Anne Debeer,
University Hospitals Leuven, Belgium

***Correspondence:**
Katrin B. Zahn
katrin.zahn@umm.de

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Objective: After neonatal repair of congenital diaphragmatic hernia (CDH) recurrence is the most severe surgical complication and reported in up to 50% after patch implantation. Previous studies are difficult to compare due to differences in surgical techniques and retrospective study design and lack of standardized follow-up or radiologic imaging. The aim was to reliably detect complication rates by radiologic screening during longitudinal follow-up after neonatal open repair of CDH and to determine possible risk factors.

Methods: At our referral center with standardized treatment algorithm and follow-up program, consecutive neonates were screened for recurrence by radiologic imaging at defined intervals during a 12-year period.

Results: 326 neonates with open CDH repair completed follow-up of a minimum of 2 years. 68 patients (21%) received a primary repair, 251 (77%) a broad cone-shaped patch, and 7 a flat patch (2%). Recurrence occurred in 3 patients (0.7%) until discharge and diaphragmatic complications in 28 (8.6%) thereafter. Overall, 38 recurrences and/or secondary hiatal hernias were diagnosed (9% after primary repair, 12.7% after cone-shaped patch; $p = 0.53$). Diaphragmatic complications were significantly associated with initial defect size ($r = 0.26$). In multivariate analysis left-sided CDH, an abdominal wall patch and age below 4 years were identified as independent risk factors. Accordingly, relative risks (RRs) were significantly increased [left-sided CDH: 8.5 ($p = 0.03$); abdominal wall patch: 3.2 ($p < 0.001$); age ≤4 years: 6.5 ($p < 0.002$)]. 97% of patients with diaphragmatic complications showed no or nonspecific symptoms and 45% occurred beyond 1 year of age.

Conclusions: The long-term complication rate after CDH repair highly depends on surgical technique: a comparatively low recurrence rate seems to be achievable in large defects by implantation of a broad cone-shaped, non-absorbable patch. Longitudinal follow-up with regular radiologic imaging until adolescence is essential to reliably detecting recurrence to prevent acute incarceration and chronic gastrointestinal morbidity with their impact on prognosis. Based on our findings and literature review, a risk-stratified approach to diaphragmatic complications is proposed.

Keywords: congenital diaphragmatic hernia, CDH, recurrence, secondary hiatal hernia, radiologic screening, longitudinal follow-up, risk factors for recurrence, cone-shaped patch

INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a rare malformation, and surgical repair is still an intervention with a remarkable complication rate. High-risk patients are nowadays already identified on prenatal investigation (1–3). It has been shown that these are more likely to require postnatal extracorporeal membrane oxygenation (ECMO) therapy and diaphragmatic reconstruction with a patch and that they are at risk of early mortality and long-term morbidity (4). These fetuses should therefore be transferred to a high-volume center for optimized treatment and follow-up (5). Improvements in pre-, peri-, and postnatal care have enhanced survival rates, and thus long-term morbidity gains more importance (6, 7). Survivors may suffer from lung hypoplasia, pulmonary hypertension, gastrointestinal problems, failure to thrive, and orthopedic and neurological side effects (8–14). Even among high-volume centers, a great variability exists concerning patients, to whom follow-up is offered, time intervals of follow-up visits, diagnostic testing, and standardization of follow-up—with only 3 of 19 centers (16%) offering long-term follow-up to all CDH patients routinely (15). Recently, the importance of longitudinal follow-up for CDH survivors due to their numerous comorbidities and complex needs has been emphasized and a schedule for a risk-stratified multi-specialty follow-up has been proposed (16).

It has been stated that primary CDH repair might be possible in 60–70% (17). In patients with large defects, a muscle flap or synthetic patches are required as a substitute for the diaphragm (18, 19). Different absorbable and non-absorbable materials, suture techniques, and shapes of these patches have been introduced (5, 19–21). Especially in large diaphragmatic defects, the abdominal cavity is hypoplastic, because most abdominal organs herniated into the thoracic cavity, and neonates present with a collapsed abdomen. Therefore, in some cases the implantation of an abdominal wall patch may be necessary to prevent abdominal compartment syndrome and compromise of intestinal and renal perfusion.

In all techniques of diaphragmatic reconstruction, recurrence (R) is a common complication. In-hospital recurrence has been reported from the CDH registry in 2.7% in open surgery (OS) (22). Thereafter, late recurrence may slowly develop with growth and seems to be asymptomatic in most patients (23). Yet, recurrence can be the underlying cause for chronic gastrointestinal problems and failure to thrive, which can consequently cause impaired neurologic and cognitive function (12). On the other hand, recurrence can cause sudden intestinal incarceration. Gastrointestinal morbidity is the leading cause of mortality beyond the first year of life among CDH survivors (24). Also, reports on CDH as cause of severe complications and mortality in adults emphasize the importance of paying attention to this complication in childhood. Therefore, it seems essential to treat recurrence before patients encounter acute incarceration with the risk of bowel gangrene, septicemia, and death.

Reported incidences of recurrence in childhood vary between 4 and >50% depending on patient selection, surgical procedure, and patch material (25–28). A reduced recurrence rate after implantation of a cone-shaped patch was published by Loff in 2005 (29). After these promising preliminary results, a prospective standardized multidisciplinary follow-up program with regular radiological imaging was established at our institution. In the current study, we examined long-term rates of diaphragmatic complications after neonatal open CDH repair and aimed at identifying possible risk factors.

MATERIALS AND METHODS

Study Group

Consecutive neonates born January 1, 2003 to December 31, 2012, at our neonatal intensive care unit (NICU) at the Department of Neonatology of the University Children's Hospital Mannheim, University of Heidelberg, who underwent open abdominal surgery and completed follow-up for at least 2 years were included in this prospective study. Exclusion criteria were death before discharge (referred to as early mortality), minimally invasive surgery, and loss of follow-up <2 years. Death beyond discharge is referred to as late mortality. In patients who were seen at an older age and did not have a recurrence, it was postulated that they also did not have one before this time. Data were collected prospectively until January 2016. This study was approved by our local ethic committee (2018-592N-MA), and informed consent was obtained from parents.

TABLE 1 | Standardized follow-up program for children with congenital diaphragmatic hernia at our institution (time intervals and imaging/testing, ECHO, echocardiography).

	Birth	1/2 y.	1 y.	2 y.	4 y.	6 y.	10 y.	14 y.	18 y.
ECG+ECHO	X	X	X	X	X	X	X	X	X
Chest X-ray	X	X	X	–	X	X	–	X	–
MRI	–	–	–	X	–	–	X	–	–
Low–dose CT	–	–	–	–	–	–	–	–	X
Neurologic testing	–	X	X	X	X	X	–	–	–
Ophthalmologist	X	–	X	–	–	–	–	–	–
Hearing test	X	–	X	–	–	–	–	–	–
Lung function	–	–	–	–	–	X	X	X	X

Follow-Up Program

For an overview of our standardized follow-up program, please see **Table 1**. An anterior–posterior chest X-ray is performed at defined intervals to screen recurrence. In doubt, further imaging techniques may be applied. At 2 and 10 years, the diaphragm was investigated more accurately with MRI to exclude recurrence by three-dimensional imaging.

Surgical Techniques

Within the study period, different surgical approaches have been applied: primary repair was achieved in patients with sufficient diaphragm and small defect sizes by OS until 2007 and mainly by minimally invasive surgery (MIS) thereafter. Either plain or oversize patches were only used in smaller defect size not eligible for primary repair. A cone-shaped GORE-TEX® patch has been established as the standard procedure for large defects since 2003 (29). A broad cone shape is formed extracorporeally, and the patch is then implanted with an overlapping border (**Figure 1**). In OS, a median laparotomy was performed. In patients with a hypoplastic abdominal cavity requiring an abdominal wall patch for closure, an ellipsoid GORE-TEX® patch was sutured to the fascia bilaterally and the skin closed over it as far as possible after subcutaneous mobilization.

Intraoperatively, defect size was classified according to the CDH Study Group (CDH-SG) (30) since 2008.

Statistical Analysis

For data analysis, MedCalc Statistical Software version 15.8 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2015) was used. Fisher's exact test was used to test for statistical significance, because the number of expected frequencies was low. p-values < 0.05 were considered significant. Re-recurrences were handled as separate recurrences in the data analysis. In multivariable regression analysis, recurrence was the dependent variable. Possible risk factors of recurrence were identified using Fisher's exact test and then entered into multivariable regression analysis as independent variables. Afterward, relative risks (RR) and 95% confidence intervals (CI) were calculated. Rank correlation with Spearman's formula was used to test for the degree of relationship between recurrence and defect size, because the distribution of these two variables was not normal.

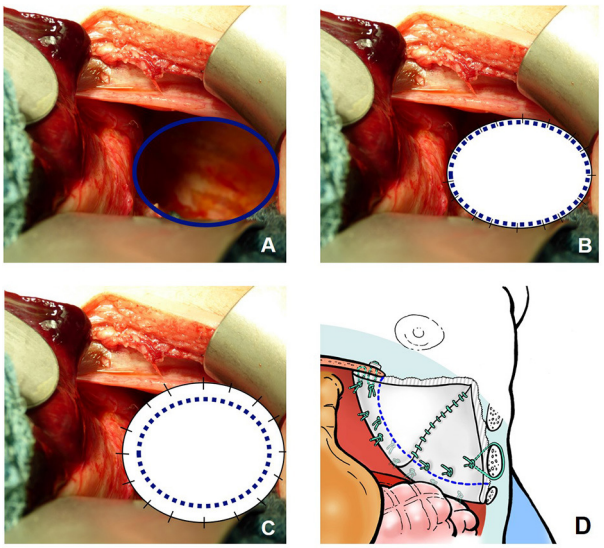

FIGURE 1 | Different surgical approaches for a diaphragmatic defect not suitable for primary repair in left-sided congenital diaphragmatic hernia (CDH): **(A)** prior to closure; **(B)** after closure with a plain patch; **(C)** after closure with an "oversize" patch; **(D)** after closure with a broad cone-shaped patch.

RESULTS

Demographic Data of the Study Cohort, Mortality, and Follow-Up

A consort diagram of the patients of our study cohort is presented in **Figure 2**. In 508 neonates with CDH born in the study period, survival to discharge was 81% ($n = 410$): 37 patients (7%) died without surgery due to prematurity, fatal syndrome or associated malformations, severe lung hypoplasia, or contraindication to ECMO therapy; 29 of the ECMO patients (14%) died without CDH repair; and 26 (13%) died after CDH repair. Early mortality was 27% in ECMO and 2% in non-ECMO patients ($p < 0.001$).

In patients who underwent CDH repair, survival was 93%: 100% in MIS patients, 97% in non-ECMO-OS patients, and 85% in ECMO patients. Survival rate in non-ECMO patients was significantly higher compared to ECMO patients ($p < 0.001$). Late mortality did not differ significantly between ECMO- and non-ECMO patients [9/131 (7%) vs. 5/195 (3%), $p = 0.09$].

Of 410 CDH patients surviving to discharge, 370 (90%) participated in our longitudinal follow-up program. Forty-four MIS patients were excluded because the aim of the study was to evaluate the complication rate and risk factors after open CDH repair. Thus, 326 patients with a minimum follow-up of 2 years were eligible for further analysis. Details of our study population are described in **Table 2**. No significant difference between OS patients with and without follow-up could be detected. There was a predominance of male neonates and left-sided CDH in our cohort. ECMO was performed in 40% of

neonates. Diaphragmatic reconstruction was achieved primarily in 21%, with a cone-shaped patch in 77% and with other patch types in 2%. In left-sided CDH, an intrathoracic liver and stomach herniation was noted in 60 and 79%, respectively. An abdominal wall patch was required in 17%. In 140 patients with intraoperative classification of defect size, large C and D defects were noted in 71%.

Thirty-eight diaphragmatic complications were detected in 31 patients within an observational time of 2–10 years. Six patients developed re-recurrences (19.3%). For further analysis, each of the re-recurrences was handled as a separate one.

Diaphragmatic Complications

We have detected two different types of diaphragmatic complications: "true" recurrence at the localization of the original diaphragmatic defect and secondary hiatal hernia. Of 38 recurrences, 24 (63%) were "true" recurrences, eight (21%) were hiatal hernias, and six (16%) patients had both (**Figure 3**).

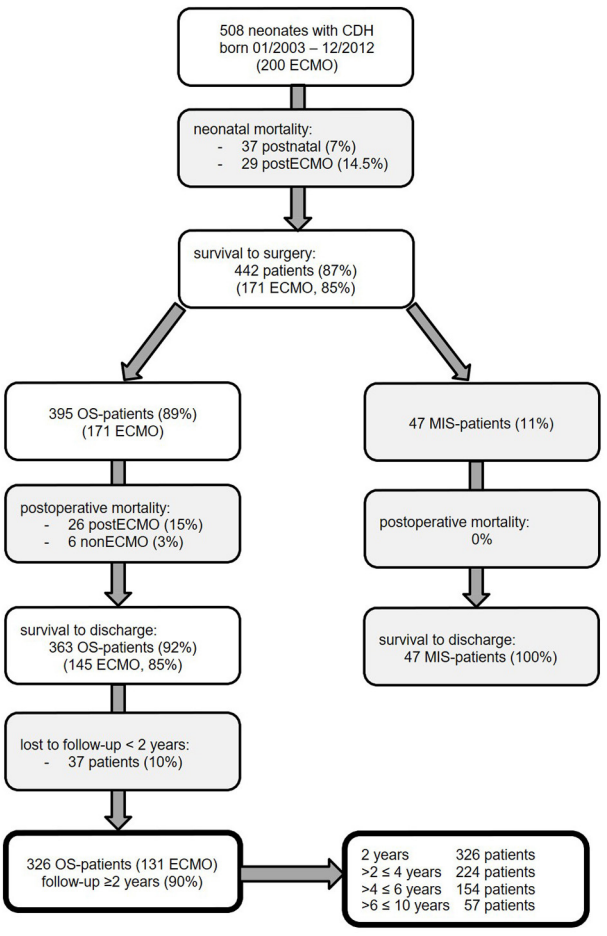

FIGURE 2 | Neonates with congenital diaphragmatic hernia (CDH) born January 2003 to December 2012 at our institution and participation at follow-up until January 2016 with excluded patients in gray boxes [ECMO, extracorporeal membrane oxygenation; MIS, minimally invasive surgery; OS, open surgery].

Patient characteristics are displayed in **Table 3**. All patients with secondary hiatal hernia and co-occurrence had an l-CDH with initial stomach herniation. Most patients had an intrathoracic herniation of the left liver lobe and required patch repair of the diaphragmatic defect, whereas a higher rate of abdominal wall patch implantation can be noticed in patients with "true" recurrence or co-occurrence. Most children who developed solely secondary hiatal hernia did not show any symptoms, while all with a co-occurrence did.

Time and Symptoms

Three of 410 patients (0.7%) surviving to discharge developed in-hospital recurrence. After discharge, 18 (51.4%) diaphragmatic complications were diagnosed within the first year of life, 11 (31.4%) within the second, three (8.6%) between 2 and 4 years of age, and three (8.6%) thereafter. Thus, the incidence of diaphragmatic complications was highest within the first year of life (21/326; 6.4%) and reduced to about half in the second year (11/326; 3.4%). In patients between 2 and 4 years of age, the incidence was 1.3% (3/224) and 1.9% (3/154) in children older than 4 years, respectively.

One patient presented with acute incarceration and intestinal obstruction (2.6%). In 35 patients (92.1%), recurrence was detected by radiologic imaging before discharge or on follow-up visits (examples in **Figure 3**) and in two (5.3%) incidentally during abdominal surgery for other reasons. These children were either asymptomatic (16/37 patients, 43.2%) or showed at least one of the following mild and non-specific symptoms: intermittent abdominal pain (14/37, 37.8%), gastroesophageal reflux (GER; 9/37 patients, 24.3%), a change in eating habits

TABLE 2 | Comparison between patients after open surgery (OS) with and without follow–up: epidemiologic data, intraoperative findings, and type of surgery are displayed [l–CDH, left-sided congenital diaphragmatic hernia; r-CDH, right-sided congenital diaphragmatic hernia; FETO, fetoscopic endotracheal occlusion; ECMO, extracorporeal membrane oxygenation].

		With follow-up ($n = 326$)	Without follow-up ($n = 37$)	*P*-value
Male, *n* (%)		191 (59)	19 (51)	0.48
Female, *n* (%)		135 (41)	18 (49)	
l-CDH, *n* (%)		262 (82)	30 (81)	1.0
r-CDH, *n* (%)		62 (17)	7 (19)	
Liver-up in l-CDH, *n* (%)		156 (60)	17 (57)	1.0
Stomach-up in l-CDH, *n* (%)		206 (79)	23 (77)	1.0
FETO, *n* (%)		24 (7)	2 (5)	1.0
ECMO, *n* (%)		131 (40)	13 (35)	0.6
Primary repair, *n* (%)		68 (21)	12 (32)	0.15
Cone-shaped patch, *n* (%)		251 (77)	25 (68)	
Abdominal wall patch, *n* (%)		55 (17)	2 (5)	0.09
Defect size (30), *n* (%) (since 2008, 140 pat. with follow-up, 16 pat. without follow-up)	A	4 (3)	2 (12)	0.12
	B	36 (26)	3 (19)	0.76
	C	84 (60)	10 (63)	1.0
	D	16 (11)	1 (6)	1.0

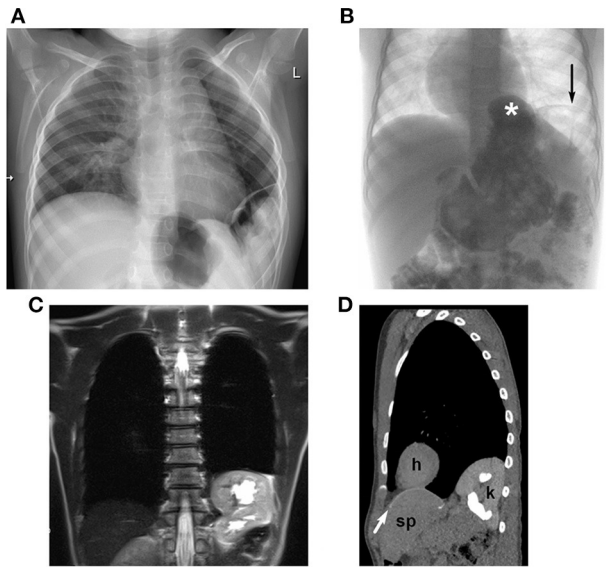

FIGURE 3 | Radiological diagnosis of recurrence of diaphragmatic hernia: **(A)** plain chest-X-ray in a 2-year-old boy: lateral recurrence; **(B)** contrast study in a 4-year-old boy: hiatal hernia (*) and lateral recurrence (→); MRI **(C)** and low-dose CT **(D)** in a 10-year-old girl with thoracic herniation of the left kidney with moderate hydronephrosis [CT scan **(D)**: h, heart; k, kidney; sp, spleen; cone-shaped patch marked with white arrow].

TABLE 3 | Patient characteristics concerning diaphragmatic complications ("true recurrence" at the localization of the original diaphragmatic defect, secondary hiatal hernia, and co-occurrence): epidemiologic data, intraoperative findings and type of surgery, symptoms, and recurrence repair rate are displayed [l-CDH, left-sided congenital diaphragmatic hernia; r-CDH, right-sided congenital diaphragmatic hernia; ECMO, extracorporeal membrane oxygenation].

	"True" recurrence ($n = 24$)	Hiatal hernia ($n = 8$)	Co-occurrence ($n = 6$)
l-CDH, *n* (%)	23 (96)	8 (100)	6 (100)
r-CDH, *n* (%)	1 (4)	0	0
Liver-up in l-CDH, *n* (%)	20 (87)	5 (63)	4 (67)
Stomach-up in l-CDH, *n* (%)	20 (87)	8 (100)	6 (100)
ECMO, *n* (%)	14 (58)	0	5 (83)
Primary repair, *n* (%)	3 (12)	2 (25)	1 (17)
Cone-shaped patch, *n* (%)	21 (88)	6 (75)	5 (83)
Abdominal wall patch, *n* (%)	11 (46)	1 (12)	3 (50)
Symptoms	14 (58)	2 (25)	6 (100)
Surgical repair	24 (100)	4 (50)	6 (100)

and stooling frequency (7/37 patients, 18.9%), and tachypnea ($n = 5/37$ patients, 13.5%). Weight at follow-up visits was not obtained routinely in the beginning of the follow-up program. Nevertheless, in those children with available data weight of recurrence patients was below the median weight of non-recurrence patients at follow-up visits in 66.2% (47/71 recurrence-patients); see **Table 4**.

Patient Characteristics and Treatment of CDH

An overview of patient characteristics and significant differences between patients with (R) and without (nonR) diaphragmatic complications is given in **Table 5**.

Concerning patient characteristics, there was a significant higher incidence of left-sided (l-)CDH in R patients. One recurrence (1.6%) was observed in 62 patients with right-sided (r-)CDH, while 37 recurrences (14.1%) were detected in 262 l-CDH-patients ($p = 0.004$). Two patients with bilateral CDH did not develop recurrence. In l-CDH, R patients had a significantly higher rate of intrathoracic herniation of the liver and stomach with 78 and 92%, respectively. In 140 patients with intraoperative size classification of the diaphragmatic defect, a significant correlation between rate of diaphragmatic complications and defect size was detected: the larger the initial defect, the higher the risk of diaphragmatic complications (correlation coefficient $r = 0.26$; $p < 0.002$; 95% CI for r 0.100–0.408; **Figure 4**). The difference between defect sizes C and D did not reach significance due to the small number of patients with defect size D (11/84 vs. 6/16; $p = 0.08$).

Regarding treatment of CDH, no differences concerning prenatal fetoscopic endotracheal occlusion (FETO) and postnatal ECMO therapy were detected between R and nonR patients. There was no significant difference in the rate of diaphragmatic complications between patients with primary reconstruction and repair with a cone-shaped patch in the total cohort—even though a significantly higher rate was detected in larger CDH defects in the subset of patients with intraoperatively classified defect size since 2008. Seven out of eight recurrences after primary repair occurred in patients born 2003–2007 and one in a patient with defect size B since 2008. This difference was not significant due to the small OS cohort with primary repair since the introduction of MIS in 2008 (7/57 vs. 1/11, $p = 1.0$). Only seven of 258 patch patients received other patch types in smaller defect size, and in none was recurrence observed. Solely non-absorbable material was used for patch implantation in this cohort.

There was a significantly higher risk of diaphragmatic complications after implantation of an abdominal wall patch ($p = 0.0003$). The abdominal wall patch clearly reflects disease severity in our cohort: 98% of patients also required a patch for diaphragmatic reconstruction—only in one patient with associated omphalocele was diaphragmatic closure achieved by primary repair. Seventy-eight percent required ECMO therapy for sufficient postnatal stabilization and 11% had undergone prenatal FETO therapy. In left-sided CDH, an intrathoracic position of the liver was detected in 89% and of the stomach in 98%. Defect size according to the CDH-study group was classified in 44 patients, and large defect sizes were predominant (A: 0%, B: 7%, C: 66%, D: 27%). Compared to patients without abdominal wall patch, the difference regarding these parameters is significant (**Table 6**).

Multivariable Analysis for Risk Factors

In multivariable regression analysis, the risk factors identified by Fisher's exact test were analyzed to verify, if they were influencing

TABLE 4 | Comparison of patients with (R) and without (nonR) diaphragmatic complications concerning weight at follow-up visits (GA, gestational age).

Follow-up visit	nonR patients GA: median 37+5 (min. 27+0, max. 42+0)			R patients GA: median 37+3 (min. 32+1, max. 40+2)			
	n	Median weight in kg	Range (min-max) in kg	*n*	Median weight in kg	Range (min-max) in kg	Weight below median of nonR patients *n* (%)
1 year	218	7.9	4.4–12.5	23	7.3	4.83–10	15 (65.2)
2 years	219	10.8	6.4–15.5	23	10	5.8–13.4	16 (69.6)
4 years	129	14	8.7–20	14	13.1	8.2–19	9 (64.3)
6 years	97	18	12.8–26	7	15.5	10.6–18	6 (85.7)
10 years	24	26.25	19.1–41.8	4	28.3	23.8–32	1 (25)

TABLE 5 | Comparison of patients with (R) and without (nonR) diaphragmatic complications in open surgery: epidemiologic data, intraoperative findings, and type of surgery are displayed [l-CDH, left-sided congenital diaphragmatic hernia; r-CDH, right-sided congenital diaphragmatic hernia; FETO, fetoscopic endotracheal occlusion; ECMO, extracorporeal membrane oxygenation].

		R ($n = 38$)	nonR ($n = 295$)	*P*-value
Male, *n* (%)		26 (68)	170 (58)	0.22
Female, *n* (%)		12 (32)	125 (42)	
l-CDH, *n* (%)		37 (97)	232 (79)	**<0.004**
r-CDH, *n* (%)		1 (3)	61 (21)	
Liver-up in l-CDH, *n* (%)		29 (78)	130 (56)	**0.01**
Stomach-up in l-CDH, *n* (%)		34 (92)	178 (77)	**0.049**
FETO, *n* (%)		5 (13)	21 (7)	0.2
ECMO, *n* (%)		19 (50)	114 (39)	0.22
Primary repair, *n* (%)		6 (16)	64 (22)	0.53
Cone-shaped patch, *n* (%)		32 (84)	224 (76)	
Abdominal wall patch, *n* (%)		15 (40)	41 (14)	**<0.001**
Defect size (30), *n* (%) (since 2008, 140 pat.)	A	0	4 (3)	1.0
	B	1 (6)	35 (28)	**0.04**
	C	11 (61)	73 (58)	1.0
	D	6 (33)	13 (10)	**0.02**

Significant p-values are given as bold values.

diaphragmatic complications independently. In all patients, CDH laterality and an abdominal wall patch were independent variables for diaphragmatic complications (multiple-correlation coefficient 0.31, CDH laterality $p = 0.03$; abdominal wall patch $p < 0.001$, F-ratio 17, $p < 0.001$). In l-CDH, an abdominal wall patch was an independent variable, while liver and stomach positions were not (multiple-correlation coefficient 0.34, "liver-up" $p = 0.07$; "stomach-up" $p = 0.53$; abdominal wall patch $p < 0.001$, F-ratio 11.5, $p < 0.001$).

Determination of RRs

The RR for diaphragmatic complications was significantly increased to 8.5 in l-CDH (95% CI 1.2–61, $p = 0.03$) and to 3.2 in patients requiring an abdominal wall patch (95% CI 1.8–5.8, $p < 0.001$). In l-CDH, the RR was 2.5-fold higher in neonates with "liver-up" (95% CI 1.2–5.3, $p = 0.01$).

A significantly increased RR could also be calculated concerning the time of diaphragmatic complications: it was 3.9-fold higher in children younger than or equal to 2 years compared to older children (95% CI 1.6–9.1, $p < 0.002$). Patients had a 6.5-fold higher risk within the first 4 years of life compared to older age (95% CI 2–20.7, $p < 0.002$).

DISCUSSION

This study demonstrated that longitudinal follow-up with regular radiologic investigation allows a reliable detection of diaphragmatic complications with the vast majority of these patients showing no or non-specific symptoms and about half occurring beyond 1 year of age. To our knowledge, it has not been explicitly mentioned by any other author before that not only recurrence at the localization of the original diaphragmatic defect but also secondary hiatal hernia is a common complication after neonatal CDH repair. Furthermore, patients with large defects are prone to develop both. In this study cohort with a predominance of large CDH, a low rate of diaphragmatic complications might have been achieved with the implantation of a broad cone-shaped, non-absorbable patch. As independent risk factors, left-sided CDH and the necessity for an abdominal wall patch could be identified in multivariate analysis.

Reports on late recurrences after OS vary strikingly between 4 and 57%, and no decline over decades can be noticed after patch repair (25, 27, 28). Multiple factors can influence recurrence: type of CDH repair, patch material, implantation technique, and various patient characteristics. Yet, it is difficult to compare results: most studies are retrospective and did not offer long-term follow-up—if any—to all surviving CDH patients, and follow-up did not regularly comprise radiologic imaging. Therefore, recurrence rates published in these studies are most likely underestimated.

Time and Symptoms

It has to be differentiated between early recurrences within the first hospital stay and late recurrences thereafter. According to the CDH registry, CDH recurred early in 2.7% of OS patients with annual recurrence rates ranging from 1.1 to 3.7% (22, 31). In our cohort, early recurrence was very rare (0.7%).

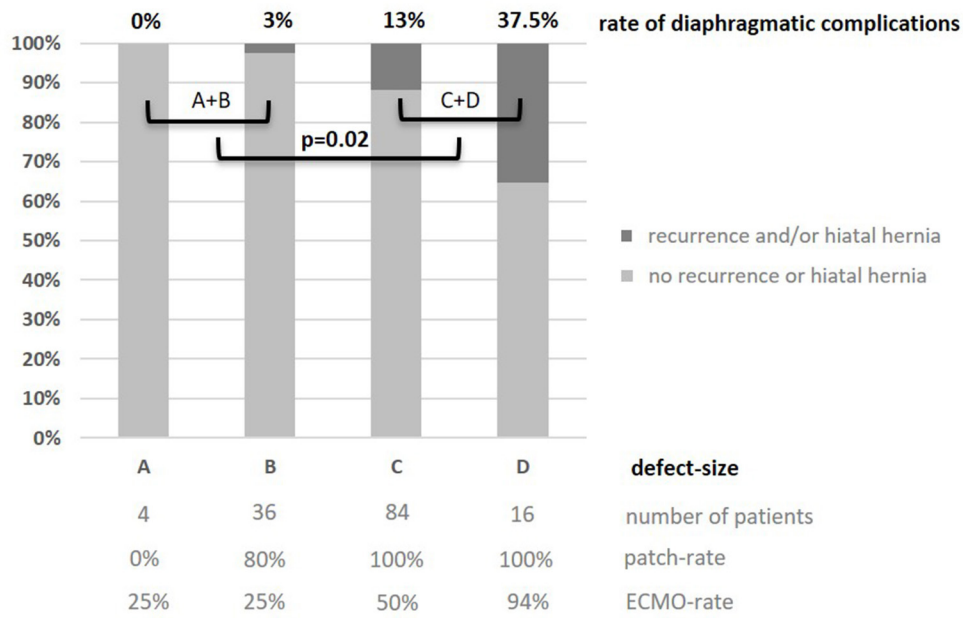

FIGURE 4 | Rate of recurrence and/or secondary hiatal hernia in relation to defect-size A-D (30) in 140 patients after open surgery 2008–2012: the larger the defect size, the higher the complication rate; significant difference between small and large defects (1/40 A+B vs. 17/100 C+D; $p = 0.02$). Additionally, patch and ECMO rates depending on defect size are displayed.

TABLE 6 | Comparison between patients with and without abdominal wall patch: intraoperative findings and type of surgery are displayed [*one patient with associated omphalocele; CDH, congenital diaphragmatic hernia; FETO, fetoscopic endotracheal occlusion; ECMO, extracorporeal membrane oxygenation].

		Abdominal wall patch ($n = 55$*)	No abdominal wall patch nonR ($n = 271$)	*P*-value
l-CDH, *n* (%)		47* (86)	215 (79)	0.26
r-CDH, *n* (%)		7 (13)	55 (2)	
Liver-up in l-CDH, *n* (%)		42* (89)	114 (53)	**<0.001**
Stomach-up in l-CDH, *n* (%)		46 (98)	160 (74)	**<0.001**
FETO, *n* (%)		6 (11)	18 (7)	0.26
ECMO, *n* (%)		43 (78)	88 (32)	**<0.001**
Primary repair, *n* (%)		1* (2)	67 (25)	**<0.001**
Cone-shaped patch, *n* (%)		54 (98)	204 (75)	
Defect size (30), *n* (%) (since 2008, 140 pat.)	A	0 (0)	4 (4)	0.31
	B	3* (7)	33 (34)	**<0.001**
	C	29 (66)	55 (57)	0.36
	D	12 (27)	4 (4)	**<0.001**

Significant p-values are given as bold values.

Recurrence after discharge has been observed within the first year in the majority of patients by several authors (23, 32–35). In our cohort, only 51% of diaphragmatic complications after discharge were diagnosed within the first year and 83% within 2 years. Consequently, 17% occurred beyond 2 years and 9% beyond 4 years of age.

With implantation of a broad cone-shaped, non-absorbable patch, and meticulous surgical technique, recurrence may develop with growth, but a lower incidence and a shift to older age could be observed in our cohort—reducing the need for secondary surgery in early infancy with its possibly negative side effect of general anesthesia on cerebral and neurologic development (36, 37).

Reports on CDH recurrence and its impact on chronic gastrointestinal morbidity and potential late mortality are limited, but there seems to be a correlation beyond the first year of life that is devastating for patients and families (24). Also, in a multivariate analysis it could be shown that mortality and the number of reoperations are significantly increased in patients with complications within 1 year after CDH repair (38). In our cohort, nonspecific symptoms associated with recurrence were mainly gastrointestinal (43.2%) and less often respiratory (13.5%). Of course, these could also be dependent on internal comorbidities of CDH and therefore be overlooked or undervalued. Accordingly, failure to thrive could also be associated with and explained by persistent pulmonary hypertension, increased respiratory effort due to lung hypoplasia, associated malformations, and adhesions. When comparing weight of R and nonR patients, it seems evident that two-thirds of R patients showed less thriving than nonR patients. However, chronic gastrointestinal problems and late mortality due to an underlying recurrence of CDH could be prevented, if diagnosed and treated timely.

Furthermore, two recently published reviews provide an insight into complications and mortality in adults with late-presenting CDH, which was thought to be a harmless situation. With less than 100 cases, left-sided CDH is rarely diagnosed in adults but seems to be correlated with a high rate of

gastrointestinal complications and mortality (39). Right-sided CDH is also a rare condition in adults with 44 patients being reported so far. Mainly, herniation of the small and large intestine has been observed—necessitating bowel resection due to intestinal ischemia or perforation in 23% and showing a mortality rate of 9% (40). In 16 of 39 patients (41%) with congenital Bochdalek hernia or CDH becoming evident during pregnancy, severe complications (intestinal obstruction, gastric gangrene, volvulus, ischemic bowel necrosis, splenic infarction, and/or cardiorespiratory failure) have led to emergency surgery (41). Alike in our patient cohort, patients in adulthood also presented with mainly gastrointestinal symptoms. There have been few reports on symptomatic hydronephrosis and/or arterial hypertension in patients with herniated kidneys that resolved after surgical repair of the diaphragmatic defect (40). Therefore, CDH containing abdominal viscera is considered to be an emergency in adults that should be repaired as soon as possible to reduce mortality and morbidity (39–41). On the other hand, the presence of a small Bochdalek hernia containing omentum or fatty tissue has been reported more frequently in CT scans performed for other reasons (42, 43). This condition is usually described as an incidental finding in asymptomatic patients and may be managed expectantly.

The apparently substantial risk of gastrointestinal morbidity and late mortality in patients with visceral (re-) herniation emphasizes the importance of a standardized follow-up program until adolescence and regular radiologic imaging also in apparently asymptomatic CDH survivors to evaluate the real long-term prevalence of recurrence and morbidity that will otherwise be unrecognized and underestimated. Furthermore, a hidden mortality may be attributed to unrecognized CDH recurrence that cannot be detected in retrospective studies and those lacking long-term follow-up. CDH is a rare malformation and pediatricians, and general practitioners looking after these patients after discharge from the hospital may not be aware of CDH recurrence as a complication, which may present with nonspecific gastrointestinal symptoms and become life-threatening within a short time after the first onset of symptoms. Alike in adulthood, the risk of morbidity and mortality is likely to be higher in patients undergoing emergency surgery—while on the other hand, these could be lowered in patients operated in an elective setting. In future, larger prospective cohort studies should be able to provide an answer to this hypothesis.

Diaphragmatic Complications

No study investigating "true" recurrence and secondary hiatal hernia has been reported so far. "True" recurrence after patch implantation can be due to pericostal sutures growing through the ribs or distraction of the patch from the hypoplastic diaphragm. It bears the risk of intestinal complications such as chronic gastrointestinal problems possibly resulting in failure to thrive with its potential negative impact on neurologic and cognitive development (12). On the other hand, acute incarceration with the risk of bowel gangrene and lethal septicemia can result (24). This can also happen after decades in undiagnosed CDH, attributing to a high risk of complications with associated mortality and morbidity (39–41). In girls, an untreated recurrence may endanger mother and child during future pregnancy. A recently published systematic review of pregnant women with diagnosis of Bochdalek hernia revealed a substantial risk of maternal and/or fetal death and preterm delivery. The incidence of bowel obstruction, ischemia, or perforation was 44%, and the risk of adverse outcome consequently increased. The authors therefore concluded that diagnosis and surgical repair should be achieved as early as possible (44). In herniated kidneys, hydronephrosis with loss of renal function and secondary arterial hypertension due to pelviureteric obstruction or compression of the renal vessels can result. Hiatal hernia is caused by distraction of the diaphragmatic crura from the esophagus especially in patients with a hypoplastic medial diaphragm and initial intrathoracic stomach herniation. It may or may not be associated with relevant GER and failure to thrive. Long-term GER may cause pulmonary compromise due to repetitive microaspirations and Barrett's esophagus at older age.

Patient Characteristics and Treatment of CDH

A predominance of CDH recurrence in r-CDH was observed by several authors—ranging from 4 to 50%—while others reported no significant difference in recurrence rate depending on CDH laterality (33, 45–48). In contrast, we observed a significantly lower recurrence rate in r-CDH. We had a similar incidence of r-CDH (19%) compared to literature reports, but a much higher patch rate—although this did not differ between r- and l-CDH in our cohort (r-CDH: 82%, l-CDH: 78.6%, $p = 0.6$). In our series, RR for diaphragmatic complications was increased significantly 8.5-fold for l-CDH. The higher incidence of recurrence in l-CDH is a consequence of intestine re-protruding intrathoracically. In r-CDH, the liver is too large and may be adherent to the patch and covering well the recurrent defect from below. Small recurrences may also develop in r-CDH but may not cause any problems and may not be detected by radiological imaging due to absent re-herniation of abdominal viscera.

The CDH-SG reported an incidence of defect sizes A and B of 50% in OS and identified larger defect size to be an independent risk factor for in-hospital recurrence in 3,332 CDH neonates (31). To date, there are no further studies reporting on defect size and recurrence rate. Almost all reports lack information about size classification, which makes reliable comparison difficult. In our OS subcohort with classification of defect size, the incidence of defect sizes A and B was only 28.6%. Being an ECMO center, mainly patients with larger defect sizes are referred for treatment, which is a potential bias but also offers the opportunity to better evaluate complication rates in more severely affected neonates. No influence of defect size on early recurrence could be identified because of its very low incidence. In-hospital recurrences might therefore rather be due to technical failure (17, 34, 49).

First, we were able to show that the risk of long-term diaphragmatic complications correlates with initial defect size and is significantly higher in larger defects. Late complications are rather caused by patient growth: either a recurrent defect at the original localization or a secondary hiatal hernia develops. Naturally, this seems more likely to happen in patients with only

a hypoplastic diaphragm: patients with defect size D were prone to develop complications in the long term, while the recurrence rate was very low in patients with defect sizes A and B. Still, a comparatively low long-term complication rate was achieved in high-risk patients in our cohort.

Regarding treatment of CDH patients, higher recurrence rates have been reported for ECMO patients and increased odds ratios were calculated (OR = 6.3 ECMO; OR = 11.2 ECMO and patch repair) (26, 45, 50), whereas others did not observe a difference between ECMO and nonECMO patients (31, 33). In our cohort, there was also no significant difference between ECMO and non-ECMO patients—even though ECMO patients had more severe CDH (diaphragmatic patch: 96% ECMO patients vs. 68% non-ECMO patients, $p < 0.000001$; abdominal wall patch: 33% ECMO patients vs. 6% non-ECMO patients, $p < 0.000001$). This finding could be explained by the fact that in our study cohort, the need for patch repair in OS was also high in non-ECMO patients—reflecting severity of CDH in an ECMO referral center. This is a potential bias but also hints at the importance of a thorough technique of patch implantation.

A higher recurrence rate after patch implantation in OS of more than 40% has been observed by several authors (26, 33, 51, 52). In a review on morbidity after CDH repair, the risk of recurrence was reported to be 3.6 times higher after open patch repair (53). Only Riehle reported a low recurrence rate of 4% in 28 patients with an oversize patch in a retrospective study with no structured follow-up (25). Thus, there might have been recurrences not detected by the authors. In our cohort, there was no significant difference regarding long-term complication rates after primary repair (9%, 68 patients) and after implantation of a cone-shaped patch (12.7%, 251 patients). Tsai reported on a similar recurrence rate for primary repair in 75 patients (4%) and repair with a dome-shaped patch in 74 patients (5.4%). All recurrences were diagnosed within the first year, while especially patients without significant lung disease are lacking long-term follow-up (34). In our cohort, the rate within the first year was 5.9% for primary and 6.2% for patch repair. The complication rate is therefore similar in both study cohorts ($p = 0.67$) even though the patch rate was significantly higher in our patient population [53.8% (Tsai) vs. 72.4%, $p < 0.001$]. Also, Heiwegen reported no difference in recurrence rate between primary repair and patch repair patients (6% both) within 1 year of follow-up in a retrospective study of 197 patients. In 39.6% of all patients, a dome-shaped patch was implanted (38). In comparison to the only prospective cohort study of 56 patch patients and a recurrence rate of 46%, our long-term complication rate of 12.7% after implantation of a broad cone-shaped patch was significantly lower ($p < 0.001$) (51).

A surrogate marker for large diaphragmatic defects is the necessity of an abdominal wall patch. Fisher first identified the implantation of an abdominal wall patch as an independent risk factor for CDH recurrence (33). Furthermore, there has been a recent publication calculating a significantly increased odds ratio of CDH recurrence within 1 year for patients requiring an abdominal wall patch (11.3, 95% CI 1.5–84.4) (38). In our patients, this was also reproducible—yet, we are the first to show that the abdominal wall patch clearly reflects disease severity: a significantly higher incidence of intrathoracic herniation of liver and stomach in left-sided CDH, need for ECMO therapy, and patch repair and larger defects were observed in these patients as compared to patients without abdominal wall patch. In our cohort, also a significantly increased, yet lower risk for diaphragmatic complications was identified (3.2; 95% CI 1.8–5.8, $p < 0.001$).

Thus, diaphragmatic complications after patch repair seem to depend on the implantation technique. In our experience, the broad cone-shaped patch allows for a flattening with growth and thus an enlarged diameter, which reduces tension on the hypoplastic diaphragm also in the long term.

Most recurrences after primary repair in our cohort were detected until 2007 and only one since 2008 in a patient with defect size B. It seems to be essential to reduce tension on the diaphragm to reduce the recurrence rate, and therefore patch implantation is now rather frequent in defect size B (80.5%). The recurrence rate also seems to depend on patch material, but available data are inconclusive: a higher recurrence risk was reported for absorbable patches as well as for non-absorbable patches, while others did not find a difference (49, 51, 54–59). Re-recurrence rates of up to 67% have been reported (28). More recently, the use of biological patches has even been disapproved due to significantly higher recurrence rates (60). In our cohort, solely non-absorbable material was used for patch implantation (Gore-Tex Dualmesh®), which might also have an impact on our low overall recurrence and re-recurrence rates.

Proposal of a Risk-Stratified Approach to Diaphragmatic Complications

An unsolved problem is the answer to the question if and when recurrence should be repaired because secondary surgery may as well be associated with morbidity. In our cohort, only one patient presented with acute incarceration, while the majority showed either no or minor and non-specific symptoms and was diagnosed by radiologic imaging during follow-up (92%). Thus, follow-up with radiologic screening offers the opportunity to detect and treat recurrence before patients encounter severe and possibly life-threatening complications. On the other hand, it also needs to be considered that radiologic imaging exposes the patient to radiation and the healthcare system to costs. Therefore, we would recommend an adapted protocol with a closer investigation within the first 2 years of life because this seems to be the high-risk period and in longer intervals afterward. Intervals for patients after open primary repair might as well be stretched. In our opinion, there is no indication for yearly investigations, which also reduces the radiation dose and costs. Furthermore, the radiation dose for the patient is reduced by follow-up in pediatric radiology departments that should be available at specialized centers.

Based on our findings and current literature review and considering the seemingly substantial risk of complications later in life, we would like to propose a risk-stratified approach to the treatment of diaphragmatic complications: boys with herniation of omentum or upper pole of the kidney may be managed expectantly with detailed counseling of parents

and ongoing follow-up, while in girls the risk of enlargement of the diaphragmatic defect and secondary herniation of the abdominal viscera during future pregnancy should also be considered. Patients with herniation of the intestine, symptomatic hydronephrosis of the herniated kidney, arterial hypertension, or relevant gastroesophageal reflux as confirmed by endoscopy and 24-h ph (impedance) testing should rather undergo secondary surgery to prevent morbidity associated with chronic diaphragmatic complications, as explained above. Regarding timing of recurrence repair, it should also be taken into consideration that the recurrent defect will become larger with ongoing growth, which can make repair more difficult. In chronic recurrence, repetitive inflammatory stimuli may cause more severe adhesions of the herniated viscera (41) and increase the risk of intraoperative laceration. Furthermore, a seemingly uncomplicated diaphragmatic hernia or recurrence can become a life-threatening condition at any time. Prior to surgery pulmonary hypertension, obstructive pulmonary compromise, and a catabolic metabolic status should be excluded or treated accordingly to reduce perioperative complications.

Limitations and Strengths

One limitation of our study is that this is not a multicenter study. However, follow-up of a homogenous patient cohort treated with a standardized surgical technique and prospective follow-up may also be considered a strength. Only seven patients received plane patches in defect sizes not eligible for primary repair, and therefore, a comparison of recurrence rates of different patch types in type C and D defects was not possible within this cohort. Therefore, we tried to put our findings into the context of literature reports after a thorough review. The number of patients older than 6 years was low, due to the fact that follow-up data were collected until 2016, so that the long-term recurrence rate until adulthood still has to be awaited. Additionally, the recruitment period comprised 10 years (2003–2012). Intraoperative classification of defect size was established from 2008 onward, reducing the number of patients, in whom risk stratification in relation to defect size was possible. However, almost all published studies lack this information so far. Future studies might therefore have the potential to verify our findings in larger patient cohorts. Also, with the introduction of MIS at our center, the number of patients with open surgical repair of smaller defect sizes decreased. Despite these limitations and in the context of published data from other centers, our findings seem relevant because they indicate that a comparatively low rate of diaphragmatic complications can be achieved in high-risk CDH patients.

CONCLUSION

This largest prospective long-term observational cohort study with a participation rate in the follow-up of 90% of surviving patients permits a reliable assessment of recurrence and secondary hiatal hernia and determination of significant risk factors. Our data indicate that the long-term rate of diaphragmatic complications highly depends on the surgical technique: a comparatively low rate could be achieved in large diaphragmatic defects by implantation of a broad cone-shaped patch. After multivariate analysis, patients with left-sided CDH and requiring an abdominal wall patch are at risk. Unlike previous reports, diaphragmatic complications occurred within the first year of life in only half of our patients. Furthermore, our findings seem to reveal that recurrence patients mostly present nonspecific gastrointestinal symptoms and failure to thrive, which can easily be misinterpreted and increase the risk of morbidity and mortality in undiagnosed CDH recurrence. This seems to underline the importance of radiologic screening during follow-up and will have to be evaluated by future studies. In future, it might be possible to internationally agree on a standardized follow-up protocol with regular radiologic imaging until adolescence for all CDH survivors as well as a risk-stratified surgical approach to recurrence to be able to prevent recurrence-related chronic gastrointestinal morbidity and acute incarceration with their impact on long-term prognosis.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because data was pseudonomized due to longitudinal follow-up and is saved in a local database. Requests to access the datasets should be directed to christel.weiss@medma.uni-heidelberg.de.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee II of the University of Heidelberg, Medical Faculty Mannheim. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

KZ had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study design, conduct, data collection, data analysis and data interpretation, and writing and revision of this manuscript were carried out by KZ. TS, NR, and LW were involved in the supervision of data collection, data interpretation, revision of the manuscript, and final approval. MW was involved in the interpretation of radiologic imaging, study conduct, and revision of the manuscript. CW contributed to the statistical plan, data analysis, and critical revision of the manuscript. All authors contributed to the article and approved the submitted version.

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A preprint of a manuscript prepared for the Lancet was uploaded on Preprints with the Lancet as part of a 12-month trial (61).

REFERENCES

1. Schaible T, Büsing KA, Felix JF, Hop WC, Zahn K, Wessel L, et al. Prediction of chronic lung disease, survival and need for ECMO therapy in infants with congenital diaphragmatic hernia: additional value of fetal MRI measurements? *Eur J Radiol.* (2012) 81:1076–82. doi: 10.1016/j.ejrad.2011.02.060
2. Debus A, Hagelstein C, Kilian AK, Weiss C, Schönberg SO, Schaible T, et al. Fetal lung volume in congenital diaphragmatic hernia: association of prenatal MR imaging findings with postnatal chronic lung disease. *Radiology.* (2013) 266:887–95. doi: 10.1148/radiol.12111512
3. Weis M, Hoffmann S, Henzler C, Weiss C, Schoenberg SO, Schaffelder R, et al. Isolated impact of liver herniation on outcome in fetuses with congenital diaphragmatic hernia - a matched-pair analysis based on fetal MRI relative lung volume. *Eur J Radiol.* (2018) 105:148–52. doi: 10.1016/j.ejrad.2018.05.024
4. Hagelstein C, Zahn K, Weidner M, Weiss C, Schoenberg SO, Schaible T, et al. Prenatal MR imaging of congenital diaphragmatic hernias: association of MR fetal lung volume with the need for postnatal prosthetic patch repair. *Eur Radiol.* (2015) 25:258–66. doi: 10.1007/s00330-014-3410-8
5. Carmichael SL, Ma C, Lee HC, Shaw GM, Sylvester KG, Hintz SR. Survival of infants with congenital diaphragmatic hernia in California: impact of hospital, clinical, and sociodemographic factors. *J Perinatol.* (2020) 40:943–51. doi: 10.1038/s41372-020-0612-6
6. Snoek KG, Reiss IK, Greenough A, Capolupo I, Urlesberger B, Wessel L, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH EURO Consortium Consensus - (2015). Update. *Neonatology.* (2016) 110:66–74. doi: 10.1159/000444210
7. Tan JK, Banton G, Minutillo C, Hall GL, Wilson A, Murray C, et al. Long-term medical and psychosocial outcomes in congenital diaphragmatic hernia survivors. *Arch Dis Child.* (2019) 104:761–7. doi: 10.1136/archdischild-2018-316091
8. Peetsold MG, Heij HA, Kneepkens CM, Nagelkerke AF, Huisman J, Gemke RJ. The long-term follow-up of patients with a congenital diaphragmatic hernia: a broad spectrum of morbidity. *Pediatr Surg Int.* (2009) 25:1–17. doi: 10.1007/s00383-008-2257-y
9. Danzer E, Gerdes M, Bernbaum J, D'Agostino J, Bebbington MW, Siegle J, et al. Neurodevelopmental outcome of infants with congenital diaphragmatic hernia prospectively enrolled in an interdisciplinary follow-up program. *J Pediatr Surg.* (2010) 45:1759–66. doi: 10.1016/j.jpedsurg.2010.03.011
10. Danzer E, Gerdes M, D'Agostino JA, Partridge EA, Hoffman-Craven CH, Bernbaum J, et al. Preschool neurological assessment in congenital diaphragmatic hernia survivors: outcome and perinatal factors associated with neurodevelopmental impairment. *Early Hum Dev.* (2013) 89:393–400. doi: 10.1016/j.earlhumdev.2012.12.009
11. Danzer E, Gerdes M, D'Agostino JA, Bernbaum J, Hoffman C, Herkert L, et al. Neurodevelopmental outcome at one year of age in congenital diaphragmatic hernia infants not treated with extracorporeal membrane oxygenation. *J Pediatr Surg.* (2015) 50:898–903. doi: 10.1016/j.jpedsurg.2015.03.040
12. Antiel RM, Lin N, Licht DJ, Hoffman C, Waqar L, Xiao R, et al. Growth trajectory and neurodevelopmental outcome in infants with congenital diaphragmatic hernia. *J Pediatr Surg.* (2017) 52:1944–8. doi: 10.1016/j.jpedsurg.2017.08.063
13. Hollinger LE, Harting MT, Lally KP. Long-term follow-up of congenital diaphragmatic hernia. *Semin Pediatr Surg.* (2017) 26:178–84. doi: 10.1053/j.sempedsurg.2017.04.007
14. Wigen RB, Duan W, Moraes TJ, Chiu PP. Predictors of long-term pulmonary morbidity in children with congenital diaphragmatic hernia. *Eur J Pediatr Surg.* (2019) 29:120–4. doi: 10.1055/s-0038-1676586
15. IJsselstijn H, Breatnach C, Hoskote A, Greenough A, Patel N, Capolupo I, et al. Defining outcomes following congenital diaphragmatic hernia using standardised clinical assessment and management plan (SCAMP) methodology within the CDH EURO consortium. *Pediatr Res.* (2018) 84:181–9. doi: 10.1038/s41390-018-0063-3
16. Hollinger LE, Buchmiller TL. Long term follow-up in congenital diaphragmatic hernia. *Semin Perinatol.* (2020) 44:151171. doi: 10.1053/j.semperi.2019.07.010
17. Losty PD. Congenital diaphragmatic hernia: where and what is the evidence? *Semin Pediatr Surg.* (2014) 23:278–82. doi: 10.1053/j.sempedsurg.2014.09.008
18. Brant-Zawadzki PB, Fenton SJ, Nichol PF, Matlak ME, Scaife ER. The split abdominal wall muscle flap repair for large congenital diaphragmatic hernias on extracorporeal membrane oxygenation. *J Pediatr Surg.* (2007) 42:1047–50; discussion 1051. doi: 10.1016/j.jpedsurg.2007.01.041
19. Saxena AK. Surgical perspectives regarding application of biomaterials for the management of large congenital diaphragmatic hernia defects. *Pediatr Surg Int.* (2018) 34:475–89. doi: 10.1007/s00383-018-4253-1
20. Morini F, Bagolan P. Surgical techniques in congenital diaphragmatic hernia. *Eur J Pediatr Surg.* (2012) 22:355–63. doi: 10.1055/s-0032-1329411
21. Tovar JA. Congenital diaphragmatic hernia. *Orphanet J Rare Dis.* (2012) 7:1. doi: 10.1186/1750-1172-7-1
22. Tsao K, Lally PA, Lally KP. Minimally invasive repair of congenital diaphragmatic hernia. *J Pediatr Surg.* (2011) 46:1158–64. doi: 10.1016/j.jpedsurg.2011.03.050
23. Jancelewicz T, Chiang M, Oliveira C, Chiu PP. Late surgical outcomes among congenital diaphragmatic hernia (CDH) patients: why long-term follow-up with surgeons is recommended. *J Pediatr Surg.* (2013) 48:935–41. doi: 10.1016/j.jpedsurg.2013.02.005
24. Burgos CM, Modée A, Öst E, Frenckner B. Addressing the cause of late mortality in infants with congenital diaphragmatic hernia. *J Pediatr Surg.* (2017) 52:526–9. doi: 10.1016/j.jpedsurg.2016.08.028
25. Riehle KJ, Magnuson DK, Waldhausen JH. Low recurrence rate after Gore-Tex/Marlex composite patch repair for posterolateral congenital diaphragmatic hernia. *J Pediatr Surg.* (2007) 42:1841–4. doi: 10.1016/j.jpedsurg.2007.07.009
26. Moss RL, Chen CM, Harrison MR. Prosthetic patch durability in congenital diaphragmatic hernia: a long-term follow-up study. *J Pediatr Surg.* (2001) 36:152–4. doi: 10.1053/jpsu.2001.20037
27. Rowe DH, Stolar CJ. Recurrent diaphragmatic hernia. *Semin Pediatr Surg.* (2003) 12:107–9. doi: 10.1016/S1055-8586(02)00020-3
28. Gasior AC, St Peter SD. A review of patch options in the repair of congenital diaphragm defects. *Pediatr Surg Int.* (2012) 28:327–33. doi: 10.1007/s00383-012-3059-9
29. Loff S, Wirth H, Jester I, Hosie S, Wollmann C, Schaible T, et al. Implantation of a cone-shaped double-fixed patch increases abdominal space and prevents recurrence of large defects in congenital diaphragmatic hernia. *J Pediatr Surg.* (2005) 40:1701–5. doi: 10.1016/j.jpedsurg.2005.07.007
30. Lally KP, Lasky RE, Lally PA, Bagolan P, Davis CF, Frenckner BP, et al. Standardized reporting for congenital diaphragmatic hernia–an international consensus. *J Pediatr Surg.* (2013) 48:2408–15. doi: 10.1016/j.jpedsurg.2013.08.014
31. Putnam LR, Gupta V, Tsao K, Davis CF, Lally PA, Lally KP, et al. Factors associated with early recurrence after congenital diaphragmatic hernia repair. *J Pediatr Surg.* (2017) 52:928–32. doi: 10.1016/j.jpedsurg.2017.03.011
32. Al-Iede MM, Karpelowsky J, Fitzgerald DA. Recurrent diaphragmatic hernia: modifiable and non-modifiable risk factors. *Pediatr Pulmonol.* (2016) 51:394–401. doi: 10.1002/ppul.23305
33. Fisher JC, Haley MJ, Ruiz-Elizalde A, Stolar CJ, Arkovitz MS. Multivariate model for predicting recurrence in congenital diaphragmatic hernia. *J Pediatr Surg.* (2009) 44:1173–9; discussion 1179–80. doi: 10.1016/j.jpedsurg.2009.02.043
34. Tsai J, Sulkowski J, Adzick NS, Hedrick HL, Flake AW. Patch repair for congenital diaphragmatic hernia: is it really a problem? *J Pediatr Surg.* (2012) 47:637–41. doi: 10.1016/j.jpedsurg.2011.11.054
35. Nagata K, Usui N, Terui K, Takayasu H, Goishi K, Hayakawa M, et al. Risk factors for the recurrence of the congenital diaphragmatic hernia-report from the long-term follow-up study of Japanese CDH study group. *Eur J Pediatr Surg.* (2015) 25:9–14. doi: 10.1055/s-0034-1395486
36. Zani A, Lamas-Pinheiro R, Paraboschi I, King SK, Wolinska J, Zani-Ruttenstock E, et al. Intraoperative acidosis and hypercapnia during thoracoscopic repair of congenital diaphragmatic hernia and esophageal atresia/tracheoesophageal fistula. *Paediatr Anaesth.* (2017) 27:841–8. doi: 10.1111/pan.13178
37. Stratmann G, May LD, Sall JW, Alvi RS, Bell JS, Ormerod BK, et al. Effect of hypercarbia and isoflurane on brain cell death and neurocognitive dysfunction in 7-day-old rats. *Anesthesiology.* (2009) 110:849–61. doi: 10.1097/ALN.0b013e31819c7140

38. Heiwegen K, van Rooij IALM, van Heijst A, de Blaauw I, Botden SMBI. Surgical complications in children with CDH: a multivariate analysis. *World J Surg.* (2020) 44:2042–8. doi: 10.1007/s00268-020-05387-4
39. Portelli M, Bugeja M, Cini C. Left-sided Bochdalek's hernia in a young adult: a case report and literature review. *Surg J.* (2021) 7:e124–6. doi: 10.1055/s-0041-1731443
40. Ramspott JP, Jäger T, Lechner M, Schredl P, Gabersek A, Mayer F, et al. A systematic review on diagnostics and surgical treatment of adult right-sided Bochdalek hernias and presentation of the current management pathway. *Hernia.* (2021). doi: 10.1007/s10029-021-02445-1. [Epub ahead of print].
41. Testini M, Girardi A, Isernia RM, De Palma A, Catalano G, Pezzolla A, et al. Emergency surgery due to diaphragmatic hernia: case series and review. *World J Emerg Surg.* (2017) 12:23. doi: 10.1186/s13017-017-0134-5
42. Mullins ME, Stein J, Saini SS, Mueller PR. Prevalence of incidental Bochdalek's hernia in a large adult population. *Am J Roentgenol.* (2001) 177:363–6. doi: 10.2214/ajr.177.2.1770363
43. Garófano-Jerez JM, López-González Jde D, Valero-González MA, Valenzuela-Barranco M. Posterolateral Bochdalek diaphragmatic hernia in adults. *Rev Ep Enferm Dig.* (2011) 103:484–91. doi: 10.4321/S1130-01082011000900009
44. Choi JY, Yang SS, Lee JH, Roh HJ, Ahn JW, Kim JS, et al. Maternal bochdalek hernia during pregnancy: a systematic review of case reports. *Diagnostics.* (2021) 11:1261. doi: 10.3390/diagnostics11071261
45. Hajer GF, vd Staak FH, Haan AF de, Festen C. Recurrent congenital diaphragmatic hernia; which factors are involved? *Eur J Pediatr Surg.* (1998) 8:329–33. doi: 10.1055/s-2008-1071226
46. Beaumier CK, Beres AL, Puligandla PS, Skarsgard ED; Canadian Pediatric Surgery Network. Clinical characteristics and outcomes of patients with right congenital diaphragmatic hernia: a population-based study. *J Pediatr Surg.* (2015) 50:731–3. doi: 10.1016/j.jpedsurg.2015.02.027
47. Collin M, Trinder S, Minutillo C, Rao S, Dickinson J, Samnakay N. A modern era comparison of right versus left sided congenital diaphragmatic hernia outcomes. *J Pediatr Surg.* (2016) 51:1409–13. doi: 10.1016/j.jpedsurg.2016.05.001
48. Duess JW, Zani-Ruttenstock EM, Garriboli M, Puri P, Pierro A, Hoellwarth ME. Outcome of right-sided diaphragmatic hernia repair: a multicentre study. *Pediatr Surg Int.* (2015) 31:465–71. doi: 10.1007/s00383-015-3695-y
49. Jawaid WB, Qasem E, Jones MO, Shaw NJ, Losty PD. Outcomes following prosthetic patch repair in newborns with congenital diaphragmatic hernia. *Br J Surg.* (2013) 100:1833–7. doi: 10.1002/bjs.9306
50. Janssen S, Heiwegen K, van Rooij IA, Scharbatke H, Roukema J, de Blaauw I, et al. Factors related to long-term surgical morbidity in congenital diaphragmatic hernia survivors. *J Pediatr Surg.* (2018) 53:508–12. doi: 10.1016/j.jpedsurg.2017.05.032
51. Jancelewicz T, Vu LT, Keller RL, Bratton B, Lee H, Farmer D, et al. Long-term surgical outcomes in congenital diaphragmatic hernia: observations from a single institution. *J Pediatr Surg.* (2010) 45:155–60; discussion 160. doi: 10.1016/j.jpedsurg.2009.10.028
52. Nasr A, Struijs MC, Ein SH, Langer JC, Chiu PP. Outcomes after muscle flap vs. prosthetic patch repair for large congenital diaphragmatic hernias. *J Pediatr Surg.* (2010) 45:151–4. doi: 10.1016/j.jpedsurg.2009.10.024
53. Heiwegen K, de Blaauw I, Botden SMBI. A systematic review and meta-analysis of surgical morbidity of primary versus patch repaired congenital diaphragmatic hernia patients. *Sci Rep.* (2021) 11:12661. doi: 10.1038/s41598-021-91908-7
54. Mitchell IC, Garcia NM, Barber R, Ahmad N, Hicks BA, Fischer AC. Permacol: a potential biologic patch alternative in congenital diaphragmatic hernia repair. *J Pediatr Surg.* (2008) 43:2161–4. doi: 10.1016/j.jpedsurg.2008.08.040
55. Laituri CA, Garey CL, Valusek PA, Fike FB, Kaye AJ, Ostlie DJ, et al. Outcome of congenital diaphragmatic hernia repair depending on patch type. *Eur J Pediatr Surg.* (2010) 20:363–5. doi: 10.1055/s-0030-1261939
56. Grethel EJ, Cortes RA, Wagner AJ, Clifton MS, Lee H, Farmer DL, et al. Prosthetic patches for congenital diaphragmatic hernia repair: Surgisis vs. Gore-Tex. *J Pediatr Surg.* (2006) 41:29–33. doi: 10.1016/j.jpedsurg.2005.10.005
57. Romao RLP, Nasr A, Chiu PPL, Langer JC. What is the best prosthetic material for patch repair of congenital diaphragmatic hernia? Comparison and meta-analysis of porcine small intestinal submucosa and polytetrafluoroethylene. *J Pediatr Surg.* (2012) 47:1496–500. doi: 10.1016/j.jpedsurg.2012.01.009
58. St Peter SD, Valusek PA, Tsao K, Holcomb GW III, Ostlie DJ, Snyder CL. Abdominal complications related to type of repair for congenital diaphragmatic hernia. *J Surg Res.* (2007) 140:234–6. doi: 10.1016/j.jss.2007.03.018
59. Ruhrnschopf CG, Reusmann A, Boglione M, Arbio S, Salas G, Giubergia V, et al. Biological versus synthetic patch for the repair of congenital diaphragmatic hernia: 8-year experience at a tertiary center. *J Pediatr Surg.* (2021) 56: 1957–61. doi: 10.1016/j.jpedsurg.2021.02.052
60. de Haro Jorge I, Prat Ortells J, Martín-Solé O, Munoz Fernandez E, Pertierra A, Martin-Lluis A, et al. Porcine dermal patches as a risk factor for recurrence after congenital diaphragmatic hernia repair. *Pediatr Surg Int.* (2021) 37:59–65. doi: 10.1007/s00383-020-04787-w
61. Zahn K, Schaible T, Weis M, Weiss C, Wessel L. Longitudinal follow-up with radiologic imaging is essential for detection of recurrence in patients with congenital diaphragmatic hernia – results from a large prospective cohort-study at a single referral centre. *SSRN Electron J.* (2019) doi: 10.2139/ssrn.3506144

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The Chest Radiographic Thoracic Area Can Serve as a Prediction Marker for Morbidity and Mortality in Infants With Congenital Diaphragmatic Hernia

Meike Weis[1†], Sosan Burhany[2†], Alba Perez Ortiz[2], Oliver Nowak[3], Svetlana Hetjens[4], Katrin Zahn[5], Stefan Schoenberg[1], Thomas Schaible[2] and Neysan Rafat[2]*

[1] Department of Clinical Radiology and Nuclear Medicine, University Medical Center Mannheim, University of Heidelberg, Mannheim, Germany, [2] Department of Neonatology, University Children's Hospital Mannheim, University of Heidelberg, Mannheim, Germany, [3] Department of Obstetrics and Gynecology, University Medical Center Mannheim, University of Heidelberg, Mannheim, Germany, [4] Department of Biomathematics and Medical Statistics, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany, [5] Department of Pediatric Surgery, University Children's Hospital Mannheim, University of Heidelberg, Mannheim, Germany

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Edited by:
Hannes Sallmon,
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***Correspondence:**
Neysan Rafat
neysan.rafat@umm.de

[†] These authors have contributed equally to this work

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Objective: Valid postnatal prediction parameters for neonates with congenital diaphragmatic hernia (CDH) are lacking, but recently, the chest radiographic thoracic area (CRTA) was proposed to predict survival with high sensitivity. Here, we evaluated whether the CRTA correlated with morbidity and mortality in neonates with CDH and was able to predict these with higher sensitivity and specificity than prenatal observed-to-expected (O/E) lung-to-head ratio (LHR).

Methods: In this retrospective cohort study, all neonates with CDH admitted to our institution between 2013 and 2019 were included. The CRTA was measured using the software Horos (V. 3.3.5) and compared with O/E LHR diagnosed by fetal ultrasonography in relation to outcome parameters including survival, extracorporeal membrane oxygenation (ECMO) support, and chronic lung disease (CLD).

Results: In this study 255 neonates were included with a survival to discharge of 84%, ECMO support in 46%, and 56% developing a CLD. Multiple regression analysis demonstrated that the CRTA correlates significantly with survival ($p = 0.001$), ECMO support ($p < 0.0001$), and development of CLD ($p = 0.0193$). The CRTA displayed a higher prognostic validity for survival [area under the curve (AUC) = 0.822], ECMO support (AUC = 0.802), and developing a CLD (AUC = 0.855) compared with the O/E LHR.

Conclusions: Our data suggest that the postnatal CRTA might be a better prognostic parameter for morbidity and mortality than the prenatal O/E LHR.

Keywords: congenital diaphragmatic hernia (CDH), survival, chronic lung disease (CLD), extracorporeal membrane oxygenation, O/E LHR, neonate

INTRODUCTION

Congenital diaphragmatic hernia (CDH) is characterized by failure of diaphragmatic development and thoracic herniation of abdominal organs, leading to lung hypoplasia and persistent pulmonary hypertension of the newborn (PPHN) (1). The clinical course of this malformation is variable, wherefore a reliable estimation of prognosis is of particular importance. As lung hypoplasia, besides PPHN, is an important determinant marker for mortality and morbidity in CDH patients (2), quantification of the absolute lung volume is a tool to predict prognosis (3–5).

In the past years, numerous parameters have been evaluated to predict prognosis in CDH patients. The observed-to-expected (O/E) lung-to-head ratio (LHR) diagnosed by fetal ultrasonography shows a high prognostic validity for clinical outcome (6–9). Although measuring O/E LHR has been established as standard approach, this parameter can be utilized only in patients with prenatal diagnosis of CDH. For this reason, it would be preferable to also establish a parameter for prognosis, which is dependent only on postnatal data. Estimation of the chest radiographic thoracic area (CRTA) is an alternative to assess lung volume on a chest radiograph (10, 11). Recently, it has been demonstrated that CRTA can predict survival in infants with severe CDH with high sensitivity and moderate specificity (12).

In this study, we evaluated whether the postnatal CRTA was able to predict morbidity and mortality with higher sensitivity and specificity than prenatal O/E LHR. As main morbidity outcome parameters, extracorporeal membrane oxygenation (ECMO) support and chronic lung disease (CLD) were selected.

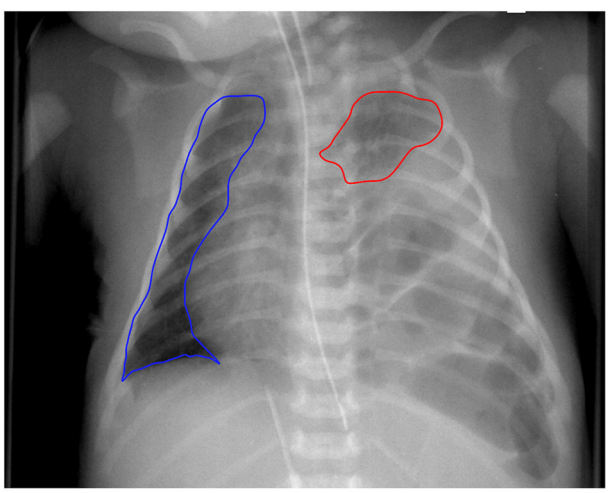


FIGURE 1 | Delineation of chest radiographic thoracic area. Example of a newborn with left-sided congenital diaphragmatic hernia. CRTA was calculated as the sum of area of ipsilateral and contralateral lung. Segmentation was performed manually.

MATERIALS AND METHODS

Subjects and Clinical Data

All newborn infants with CDH treated between January 1, 2013, and December 31, 2019, at our neonatal intensive care unit at the Department of Neonatology of the University Children's Hospital Mannheim, University of Heidelberg, were included in this retrospective study. Exclusion criteria were outborn patients; preterm infants <34 weeks' gestational age; patients with associated anomalies, syndromes, or chromosomal aberrations; newborns who died shortly after birth due to a different

TABLE 1 | Patient characteristic of the study population ($n = 255$).

Prenatal data	
Left-sided defect, n (%)	225 (88)
Liver herniation, n (%)	159 (65)
LHR	1.60 (0.5–4.6)
O/E LHR, %	38 (21–83)
rLV, %	33.5 (9–94.4)
FETO, n (%)	7 (3)
Demographics and birth	
Male sex, n (%)	145 (57)
GA, weeks	38 (34–40.3)
Birth weight, g	3,030 (1,420–4,600)
Apgar score after 5 min	8 (0–10)
CRTA, mm^2	1,155 (254–3,225)
Ventilation and additive therapies	
oxygenation index	13.9 (1.1–83.3)
HFOV, n (%)	104 (41)
HFOV on day 1, n (%)	63 (25)
Duration of mechanical ventilation, days	19.1 (0.1–214)
iNO, n (%)	178 (70)
iNO on day 1, n (%)	154 (63)
ECMO, n (%)	118 (46)
Duration of ECMO, days	9 (1.1–20.1)
Surgical	
Operated, n (%)	229 (98)
Patch repair, n (%)	189 (83)
Days to full enteral feeding	25 (0.1–178)
Outcome	
Days of hospitalization	42 (0.1–391)
Discharge with oxygen therapy (Fio_2 >0.21), n (%)	1 (1)
Discharge with HFNC, n (%)	5 (3)
Discharge with home mechanical ventilation, n (%)	4 (2)
CLD, n (%)	117 (56)
Mild, n	78 (75)
Moderate, n	17 (16)
Severe, n	9 (9)
Survival to discharge, n (%)	213 (84)

Data are expressed as median (interquartile range) or relative frequency (%).
CLD, chronic lung disease; CRTA, chest radiographic thoracic area; ECMO, extracorporeal membrane oxygenation; FETO, fetoscopic endoluminal tracheoscopic occlusion; HFOV, high-frequency oscillation ventilation; iNO, inhaled nitric oxide; LHR, lung-to-head ratio; O/E, observed-to-expected; rLV, relative lung volume.

TABLE 2 | Comparison of patient characteristics in relation to survival.

	Survived ($n = 213$)	Deceased ($n = 42$)	p-value
Prenatal data			
Left-sided defect	87% (186/213)	93% (39/42)	0.434
Liver herniation	61% (126/208)	87% (33/38)	0.002
LHR	1.7 (0.5–4.6)	1.4 (0.9–3.2)	0.002
rLV, %	35 (18–94.4)	24.2 (9–50)	<0.0001
FETO	1% (3/212)	10% (4/42)	0.016
Demographics and birth			
Male sex	58% (124/213)	50% (21/42)	0.326
GA, weeks	38 (34–40.3)	38 (34–39.6)	0.119
Birth weight, g	3,100 (2,000–4,600)	2,855 (1,420–3,500)	0.004
Apgar score after 5 min	8 (4–10)	7 (0–9)	<0.0001
Ventilation and additive therapies			
Oxygenation index	9.4 (1.1–83.3)	24.4 (4.1–54.5)	<0.0001
HFOV	33% (70/213)	81% (34/42)	<0.0001
HFOV on day 1	19% (39/210)	60% (24/40)	<0.0001
iNO	65% (138/213)	95% (40/42)	<0.0001
iNO on day 1	58% (119/205)	90% (35/39)	0.0002
ECMO	39% (84/213)	81% (34/42)	<0.0001

Data are expressed as median (interquartile range) or relative frequency (%).
ECMO, extracorporeal membrane oxygenation; FETO, fetoscopic endoluminal tracheoscopic occlusion; GA, gestational age; HFOV, high-frequency oscillation ventilation; iNO, inhaled nitric oxide; LHR, lung-to-head ratio; rLV, relative lung volume.

TABLE 3 | Comparison of patient characteristics in relation to ECMO support.

	No ECMO ($n = 137$)	ECMO ($n = 118$)	p-value
Prenatal data			
Left-sided defect	95% (130/137)	81% (95/118)	0.0004
Liver herniation	43% (66/131)	90% (103/115)	<0.0001
LHR	1.9 (1.1–4.6)	1.4 (0.5–3.2)	<0.0001
rLV, %	38.3 (20–94.4)	28.6 (9–84.1)	<0.0001
FETO	1% (1/136)	5% (6/118)	0.052
Demographics and birth			
Male sex	55% (75/137)	59% (70/118)	0.462
GA, weeks	38.1 (34.7–40.3)	38 (34–39.9)	0.1
Birth weight, g	3,100 (1,420–4,375)	2,960 (1,800–4,600)	0.502
Apgar score after 5 min	8 (0–10)	8 (4–10)	<0.0001
Ventilation and additive therapies			
Oxygenation index	5.4 (1.5–83.3)	21.6 (1.1–83.3)	<0.0001
HFOV	18% (25/137)	67% (79/118)	<0.0001
HFOV on day 1	11% (15/135)	42% (48/115)	<0.0001
iNO	44% (60/137)	100% (118/118)	<0.0001
iNO on day 1	38% (60/137)	100% (118/118)	<0.0001

Data are expressed as median (interquartile range) or relative frequency (%).
ECMO, extracorporeal membrane oxygenation; FETO, fetoscopic endoluminal tracheoscopic occlusion; GA, gestational age; HFOV, high-frequency oscillation ventilation; iNO, inhaled nitric oxide; LHR, lung-to-head ratio; rLV, relative lung volume.

complication; and patients for which an initial radiograph was missing (**Supplementary Figure 1**).

Demographic, prenatal and perinatal, and clinical data were collected from the patient's records. Estimation of CDH disease severity was based on prenatal diagnostic measures including fetal ultrasonography for liver position and measurement of observed to expected (O/E) LHR (13). The diagnosis of CLD was made as reported before (9, 14): if there was an additional need for oxygen supplementation at day 28 after birth, CLD was diagnosed. Severity of CLD was differentiated into three grades according to the additional need for oxygenation at day 56 after birth: mild CLD with no need for supplemental inspired oxygen (fraction of inspired oxygen [FIO_2] $\leq$ 0.21), moderate CLD (FIO_2, 0.22–0.29), and severe CLD ($FIO_2 \geq 0.30$). This study was approved by the local ethics committee of the Medical Faculty Mannheim of the University of Heidelberg (reference no. 2020-802R).

Chest Radiographic Thoracic Area

To assess the CRTA, the first preoperative chest radiograph of the neonates with CDH in the first 24 h after birth was included in the analysis. Chest x-rays were obtained in supine position with the tube 1 m above the patient. Lung borders were delineated manually using dedicated software (Horos, V. 3.3.5; Nimble Co. LLC d/b/a Purview in Annapolis, MD, USA) and a freehand tool, applying the following criteria (**Figure 1**): (a) contralateral: following border of the heart → delineation of the diaphragm → delineation of the thoracic wall → delineation of the upper mediastinum; (b) ipsilateral: delineation of hypoplastic lung. Areas of contralateral and ipsilateral lung were summed up to calculated CRTA. Correction of magnification error was performed by division with the factor 1.04.

Statistical Analysis

Statistical analysis was performed with SAS® version 9.4 (SAS Institute Inc., USA). Descriptive statistics were used to describe the demographic characteristics of the patients and to analyze the distribution of survival, ECMO support, and development of CLD. For metric variables, position and scatter measures, as well as distribution, were calculated. Nominal and ordinal variables were characterized using absolute and relative frequencies. The distribution of the variables was verified using the Shapiro-Wilk test. Inductive statistics was applied to evaluate the observed relationships and differences from descriptive statistics for their statistical significance. Differences in a metric characteristic between two unrelated groups were examined using the Mann–Whitney U test. For nominal or ordinal characteristics, the χ^2 test or Fisher exact test was used accordingly. In order to analyze the correlation between two metric variables, a correlation analysis according to Pearson was carried out for normally distributed data and a correlation analysis according to Spearman for non–normally distributed data. The influence of various independent parameters on survival, ECMO support, and development of CLD was analyzed in simple regression models. Subsequently, by means of step-by-step selection, significant variables were included in a multiple regression model. With the parameters O/E LHR and CRTA, logistic regression models were prepared for the respective endpoints. The significance of the parameters was assessed with receiver operating characteristic

TABLE 4 | Comparison of patient characteristics in relation to CLD.

	No CLD ($n = 91$)	CLD ($n = 117$)	p-value
Prenatal data			
Left-sided defect	92% (84/91)	83% (97/117)	0.454
Liver herniation	38% (34/89)	83% (95/115)	<0.0001
LHR	2.0 (1.1–3.5)	1.5 (0.5–3.2)	<0.0001
rLV, %	41 (22.4–94.4)	30.7 (18–65)	<0.0001
FETO	0% (0/91)	3% (4/117)	0.133
Demographics and birth			
Male sex	59% (54/91)	58% (68/117)	0.859
GA, weeks	38.1 (34.9–40.3)	38 (34–40.1)	0.155
Birth weight, g	3,120 (2,000–4,400)	2,960 (2,050–4,600)	0.084
Apgar score after 5 min	8 (4–10)	8 (4–10)	<0.0001
Ventilation and additive therapies			
Oxygenation index	3.6 (1.5–30)	17.9 (1.1–83.3)	<0.0001
HFOV	15% (14/91)	51% (60/117)	<0.0001
HFOV on day 1	9% (8/90)	27% (31/115)	0.0011
iNO	32% (29/91)	92% (109/117)	<0.0001
iNO on day 1	27% (24/90)	86% (94/110)	<0.0001
ECMO	6% (5/91)	72% (84/117)	<0.0001

Data are expressed as median (interquartile range) or relative frequency (%).
CLD, chronic lung disease; ECMO, extracorporeal membrane oxygenation; FETO, fetoscopic endoluminal tracheoscopic occlusion; GA, gestational age; HFOV, high-frequency oscillation ventilation; iNO, inhaled nitric oxide; LHR, lung-to-head ratio; rLV, relative lung volume.

(ROC) analysis by calculating the area under the curve (AUC). A cutoff value was determined using the Youden index. In a final step, the correlation of the O/E LHR or CRTA was evaluated with the duration of hospitalization or the duration of mechanical ventilation. Linear regression models were created, and their quality was evaluated by calculating coefficients of determination. $p \leq 0.05$ was considered significant.

RESULTS

Demographic and Clinical Characteristics of the Study Cohort

Between January 2013 and December 2019, 403 neonates with CDH were treated at our center, of whom 255 neonates were included in this study. For an overview of the recruitment of CDH patients into this study and the characteristics of the dropouts, see **Supplementary Figure 1**. Of the included study population, 40 patients were transferred to hospitals in the patient's home area after stabilization, surgical repair, and successful weaning. In these patients, the entire duration of the inpatient treatment could not be assessed. For an overview of the characteristics of the study population, refer to **Table 1**.

Correlation of Clinical Parameters With Survival, ECMO Support, and CLD

We first correlated several major clinical parameters with survival, ECMO support, and CLD in our study population. The neonates who died showed prenatally a significant lower O/E LHR and a lower relative lung volume (rLV) compared with the survivors (**Table 2**). They also revealed more frequently a liver herniation and received more often fetoscopic endoluminal tracheal occlusion (FETO) (**Table 2**). On day 1 of life and thereafter, a treatment with HFOV, inhaled nitric oxide (iNO), or ECMO was significantly more often established in this population (**Table 2**).

The patient population requiring ECMO support also showed prenatally a significant lower O/E LHR and a lower rLV prenatally compared with the patients without ECMO support (**Table 3**). In this patient population, a left-sided CDH and a liver herniation were more frequently present (**Table 3**). Also, in this population on day 1 of life and thereafter, a treatment with HFOV, iNO, or ECMO was significantly more often established (**Table 3**).

When comparing patients with and without CLD, patients with CLD showed prenatally a significant lower O/E LHR and a lower rLV prenatally compared with the patients without CLD (**Table 4**). Also, in this patient population, a left-sided CDH and a liver herniation were more frequently present (**Table 4**). In patients with CLD on day 1 of life and thereafter, a treatment with HFOV or iNO was significantly more often established (**Table 4**).

Correlation of O/E LHR With CRTA

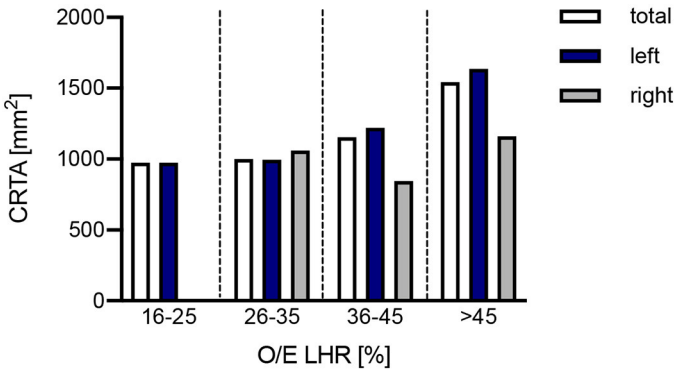
The correlation of O/E LHR and CRTA was assessed by Spearman correlation analysis. In the total study population [$r_s(168) = 0.406$, $p < 0.0001$] and in the population of patients with left-sided CDH [$r_s(147) = 0.473$, $p < 0.0001$], a significant correlation could be demonstrated for both parameters. Patients with right-sided CDH showed no significant correlation with the two parameters [$r_s(21) = 0.195$, $p = 0.396$; **Figure 2**]. The resulting values from the linear regression analysis to display the correlation of O/E LHR and CRTA are shown in **Figure 2**.

Correlation of Independent Clinical Parameters With CRTA

The impact of independent clinical parameter on the CRTA was analyzed by correlation analysis. O/E LHR ($r = 0.388$, $p < 0.0001$; **Figure 3A**), rLV ($r = 0.544$, $p < 0.0001$; **Figure 3B**), and birth weight ($r = 0.150$, $p = 0.017$; **Figure 3**) correlate significantly with CRTA, but not gestational age ($r = 0.101$, $p = 0.107$; **Figure 3C**). Neonates that were treated with high-frequency oscillation on day 1 of life had a significantly lower CRTA (929 mm^2 [254–2,832]) compared with neonates treated only with conventional ventilation therapy (1,266 [374–3,225], $p < 0.0001$) (data not shown).

Prognostic Value of O/E LHR and CRTA for Survival

The evaluation of O/E LHR between deceased and surviving neonates showed a significant difference (33.0% [21.0%−70.0%] vs. 39.0% [21.0%−83.0%], $p = 0.002$; **Figure 4A**). To assess the association of O/E LHR with mortality, a logistical regression analysis was performed, and the following regression equation



Group	r_s	*p-value*	linear regression equation	r^2
Total	0,406	<0,0001	$Y_{CRTA} = 226{,}24035 * O/E\ LHR + 348{,}60960$	0,160
Left-sided	0,473	<0,0001	$Y_{CRTA} = 262{,}22813 * O/E\ LHR + 260{,}05268$	0,216
Right-sided	0,195	0,396	$Y_{CRTA} = 116{,}25419 * O/E\ LHR + 512{,}76346$	0,029

FIGURE 2 | Correlation of observed-to-expected lung-to-head ratio with CRTA. The correlation of observed-to-expected lung-to-head ratio and CRTA is shown in the total study population [$r_s(168) = 0.406$, $p < 0.0001$], in the group of left-sided [$r_s(147) = 0.473$, $p < 0.0001$] and right-sided CDH [$r_s(21) = 0.195$, $p = 0.396$]. CRTA, chest radiographic thoracic area; LHR, lung-to-head ratio; O/E, observed-to-expected; r_s, correlation coefficient; r^2, determination coefficient.

describes the mortality rate depending on O/E LHR:

$$P_{mortality} = \frac{e^{(0.8081-0.05943*O/E\ LHR)}}{1 + e^{(0.8081-0.05943*O/E\ LHR)}}$$

An increasing O/E LHR is associated with lower mortality (**Figure 4B**). Therefore, the probability of neonates to die with an O/E LHR of 60% is 5%, whereas the probability to die with an O/E LHR of 15% is 45%. The ROC curve to predict survival showed an AUC of 0.674 (**Figure 4C**). When using 36% as a cutoff, the O/E LHR had a sensitivity of 63% and a specificity of 67% for the prediction of mortality.

Also, for CRTA, a significant difference was revealed between deceased and surviving neonates (682 mm^2 [254–1,919 mm^2] vs. 1,252 mm^2 [434–3,225 mm^2], $p < 0.0001$; **Figure 4D**). The association of CRTA and mortality could be described by the following regression equation:

$$P_{mortality} = \frac{e^{(1.807-0.003376*CRTA)}}{1 + e^{(1.807-0.003376*CRTA)}}$$

Consistent with O/E LHR, an increasing CRTA is also associated with lower mortality (**Figure 4E**); for example, the probability to die with a CRTA of 1,800 mm^2 is <1%, whereas the probability to die with a CRTA of 200 mm^2 is 75%. The ROC curve indicated an AUC of 0.822 (**Figure 4F**). When using 806 mm^2 as a cutoff, the CRTA had a sensitivity of 88% and a specificity of 69% for the prediction of mortality.

In the univariate analysis, the parameters O/E LHR, CRTA, LHR, rLV, liver herniation, FETO, birth weight, 5-min Apgar, HFOV, HFOV on day 1, iNO, iNO on day 1, oxygenation index, and ECMO were identified to be statistically significant for survival. The parameters oxygenation index, 5-min Apgar, CRTA, and FETO were gradually introduced into a multiple logistic regression model, and it could be demonstrated that CRTA ($p = 0.001$) and 5-min Apgar score ($p = 0.019$) were independently associated with survival (AUC = 0.895) (data not shown).

To evaluate whether CRTA plays an important role in certain patient populations, we performed subgroup analysis for left-sided ($n = 225$) and right-sided CDH ($n = 30$). The highest prognostic validity of O/E LHR for survival could be seen in left-sided CDH, whereas for CRTA, it was seen in right-sided CDH (**Supplementary Table 1**). **Supplementary Table 1** gives an overview of the significant parameter of the univariate and multivariate analyses for survival in regard to defect side.

Prognostic Value of O/E LHR and CRTA for ECMO Support

The evaluation of O/E LHR between neonates with ECMO support and without showed a significant difference (34.6% [21.0–78.0%] vs. 42.5% [23.0–83.0%], $p < 0.0001$; **Figure 5A**). The association of O/E LHR and ECMO support can be described

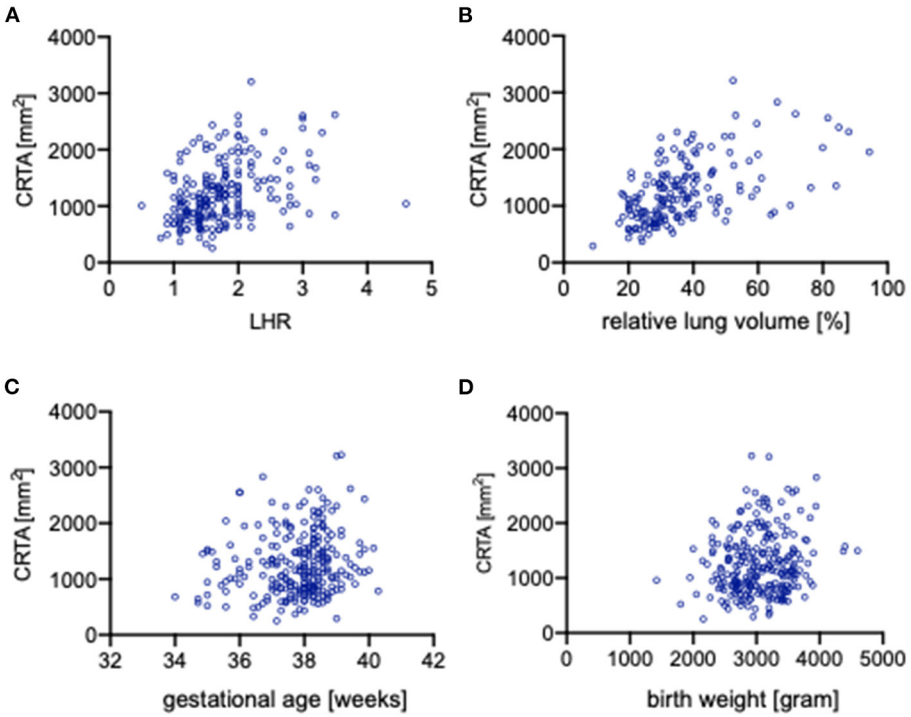


FIGURE 3 | Correlation of independent clinical parameters with CRTA. Scatterplots portraying the correlation of CRTA to **(A)** lung-to-head ratio ($r = 0.388$, $p < 0.0001$), **(B)** relative lung volume ($r = 0.544$, $p < 0.0001$), **(C)** gestational age ($r = 0.101$, $p = 0.107$), and **(D)** birth weight ($r = 0.15$, $p = 0.017$). CRTA, chest radiographic thoracic area; LHR, lung-to-head ratio.

by the following regression equation:

$$P_{ECMO} = \frac{e^{(1.971 - 0.04615 * \frac{O}{E}\ LHR)}}{1 + e^{(1.971 - 0.04615 * \frac{O}{E}\ LHR)}}$$

An increasing O/E LHR is associated with lower ECMO support (**Figure 5B**). Neonates with a prenatal O/E LHR of 15% have a probability of <75% to require ECMO support, whereas neonates with an O/E LHR of 60% have a probability of <30% of cases to require ECMO support. The ROC analysis indicated an AUC of 0.678 (**Figure 5C**). When using 39% as a cutoff, the O/E LHR had a sensitivity of 69% and a specificity of 61% for the prediction of ECMO support.

Also, for CRTA, a significant difference was revealed between neonates with ECMO support and without (875 mm^2 [254–2,258 mm^2] vs. 1,470 mm^2 [437–3,225 mm^2], $p < 0.0001$; **Figure 5D**). The association of CRTA and ECMO support could be described by the following regression equation:

$$P_{ECMO} = \frac{e^{(3.019 - 0.002642 * CRTA)}}{1 + e^{(3.019 - 0.002642 * CRTA)}}$$

Consistent with O/E LHR, an increasing CRTA is also associated with lower ECMO support (**Figure 5E**). Neonates with a CRTA of 2,000 mm^2 have a probability of <10% to require ECMO support, whereas neonates with a CRTA of 600 mm^2 have a probability of 80% to require ECMO support. The ROC curve indicated an AUC of 0.802 (**Figure 5F**). When using 1,188 mm^2 as a cutoff, the CRTA had a sensitivity of 78% and a specificity of 70% for the prediction of ECMO support.

In the univariate analysis, the parameters O/E LHR, CRTA, LHR, rLV, defect side, liver herniation, 5-min Apgar, HFOV, HFOV on day 1, iNO, iNO on day 1, and oxygenation index were identified to correlate significantly with ECMO support. The parameters iNO, iNO on day 1, and oxygenation index were gradually introduced into a multiple logistic regression model, and it could be demonstrated that iNO on day 1 ($p = 0.001$) and the oxygenation index ($p = 0.001$) were independently associated with ECMO support (AUC = 0.933) (data not shown).

In the subgroup analysis in regard to defect side, the highest prognostic validity of O/E LHR for ECMO support could be seen in left-sided CDH, whereas for CRTA, it was seen in right-sided CDH (**Supplementary Table 2**). **Supplementary Table 2** gives an overview of the significant parameters of the univariate and multivariate analysis for ECMO support in regard to defect side.

Prognostic Value of O/E LHR and CRTA for CLD

The evaluation of O/E LHR between neonates with CLD and without CLD showed a significant difference (36.0% [21.0%−78.0%] vs. 45.0% [27.0%−74.0%], $p = 0.0002$; **Figure 6A**). The association of O/E LHR and CLD can be

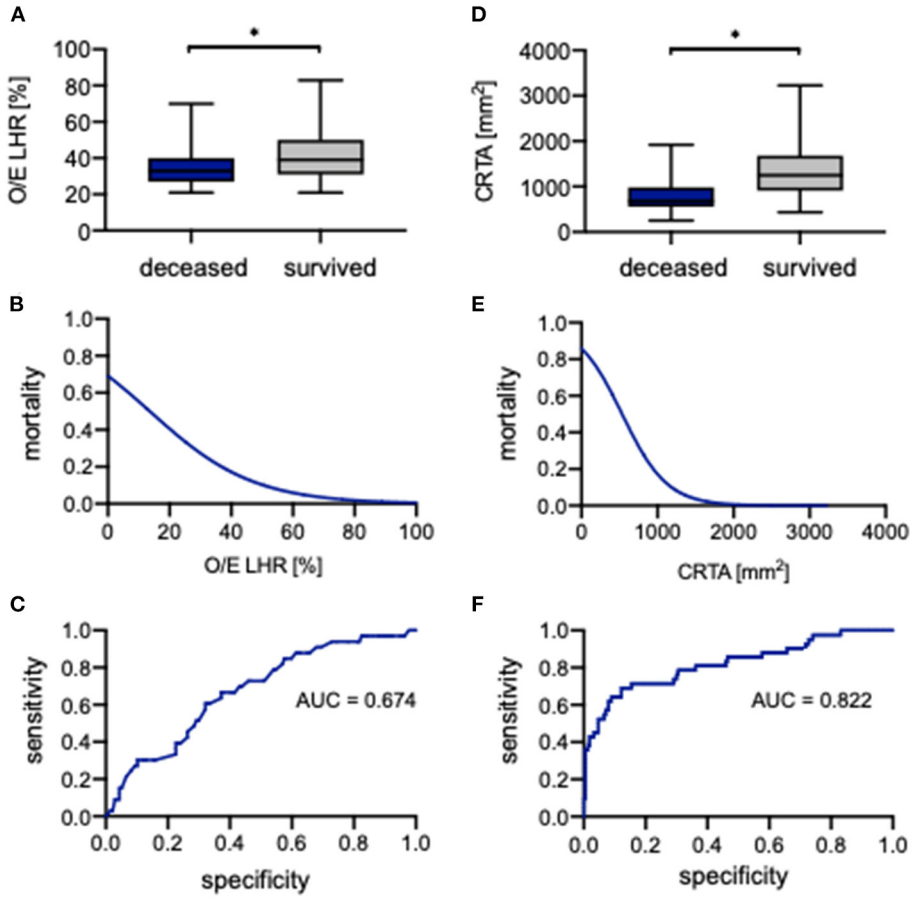


FIGURE 4 | Correlation of observed-to-expected lung-to-head ratio and CRTA to survival. Boxplots of **(A)** observed-to-expected lung-to-head ratio and **(D)** CRTA are exhibited for deceased and surviving infants (whiskers are shown as minimum and maximum). Also shown is the logistical regression **(B,E)** and ROC curve **(C,F)** of observed-to-expected lung-to-head ratio and CRTA for the prediction of mortality in the study population. *Significant difference in the *U* test. AUC, area under curve; CRTA, chest radiographic thoracic area; LHR, lung-to-head ratio; O/E, observed-to-expected.

described by the following regression equation:

$$P_{CLD} = \frac{e^{(2.626 - 0.05180 * \frac{O}{E}\ LHR)}}{1 + e^{(2.626 - 0.05180 * \frac{O}{E}\ LHR)}}$$

An increasing O/E LHR is associated with lower incidence of CLD (**Figure 6B**). Neonates with a prenatal O/E LHR of 15% have a probability of 85% to develop CLD, whereas a neonate with an O/E LHR of >60% has a probability of only 10% to develop CLD. The ROC analysis indicated an AUC of 0.700 (**Figure 6C**). When using 39% as a cutoff, the O/E LHR had a sensitivity of 68% and a specificity of 71% for the prediction of developing CLD.

Also for CRTA, a significant difference was revealed between neonates with CLD and without CLD (997 mm^2 [434–2,379 mm^2] vs. 1,610 mm^2 [787–3,225 mm^2], $p < 0.0001$; **Figure 6D**). The association of CRTA and CLD could be described by the following regression equation:

$$P_{CLD} = \frac{e^{(4.653 - 0.003318 * CRTA)}}{1 + e^{(4.653 - 0.003318 * CRTA)}}$$

Consistent with O/E LHR, an increasing CRTA is also associated with lower incidence of CLD (**Figure 6E**). Neonates with a CRTA of 1,000 mm^2 have a probability of 80% to develop CLD, whereas neonates with a CRTA of 2,000 mm^2 have a probability of <10% to develop CLD. The ROC curve indicated an AUC of 0.855 (**Figure 6F**). When using 1,383 mm^2 as a cutoff, the CRTA had a sensitivity of 86% and a specificity of 74% for the prediction of developing CLD. However, there was no significant association of either O/E LHR or CRTA with the severity of CLD (**Supplementary Figure 2**).

In the univariate analysis, the parameters O/E LHR, CRTA, LHR, rLV, defect side, liver herniation, 5-min Apgar, HFOV, HFOV on day 1, iNO, iNO on day 1, oxygenation index, and ECMO support were identified to correlate significantly with developing CLD. The parameters ECMO support, rLV, and CRTA were gradually introduced into a multiple logistic regression model, and it could be demonstrated that CRTA ($p = 0.019$), rLV ($p = 0.009$), and ECMO support ($p = 0.003$) were independently associated with developing CLD (AUC = 0.971) (data not shown).

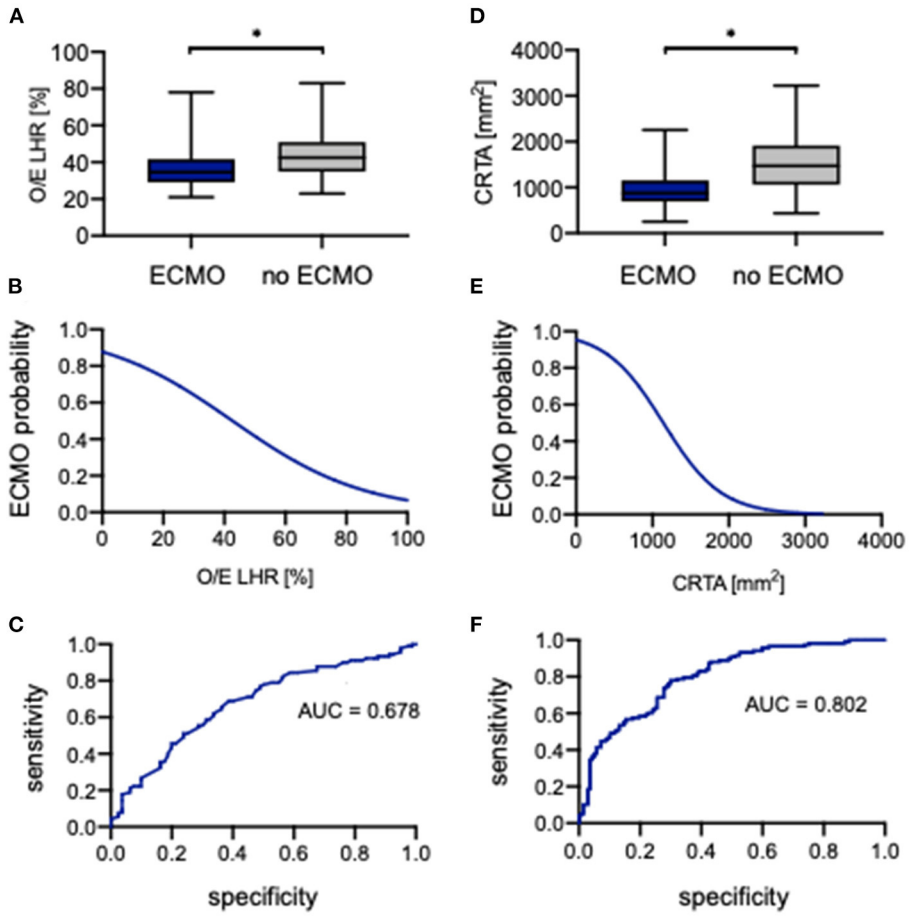


FIGURE 5 | Correlation of observed-to-expected lung-to-head ratio and CRTA to ECMO. Boxplots of **(A)** observed-to-expected lung-to-head ratio and **(D)** CRTA are exhibited for patients with ECMO and without ECMO support (whiskers are shown as minimum and maximum). Also shown is the logistical regression **(B,E)** and ROC curve **(C,F)** of observed-to-expected lung-to-head ratio and CRTA for the prediction of ECMO probability in the study population. *Significant difference in the *U* test. AUC, area under curve; CRTA, chest radiographic thoracic area; ECMO, extracorporeal membrane oxygenation; LHR, lung-to-head ratio; O/E, observed-to-expected.

In the subgroup analysis in regard to defect side, the highest prognostic validity of O/E LHR as well as CRTA for developing CLD could be seen in right-sided CDH (**Supplementary Table 3**). **Supplementary Table 3** gives an overview of the significant parameters of the univariate and multivariate analyses for developing CLD in regard to defect side.

Prognostic Value of O/E LHR and CRTA for Other Clinical Parameters

Different other clinical parameters were analyzed for their prognostic validity. In the subgroup of surviving neonates, a negative correlation of O/E LHR ($r_s = -0.438$, $p < 0.0001$; **Supplementary Figure 3A**) and CRTA ($r = -0.352$, $p < 0.0001$; **Supplementary Figure 3B**) could be demonstrated with the duration of mechanical ventilation. In the same subgroup, a negative correlation of O/E LHR ($r_s = -0.409$, $p < 0.0001$; **Supplementary Figure 3C**) and CRTA ($r = -0.465$, $p < 0.0001$; **Supplementary Figure 3D**) could be demonstrated with the duration of hospitalization.

DISCUSSION

The postnatally measured CRTA can predict important outcome parameters—survival, ECMO requirement, and the development of CLD—in children with CDH. In direct comparison to the established and prenatal parameter O/E LHR, it seems superior. The CRTA has initially been described by Dimitriou et al. (10) as a parameter that correlates well with functional lung parameters, such as the functional residual capacity.

Concerning survival, our results are in good agreement with previously published studies. Dassios et al. (12) demonstrated in a smaller patient cohort ($n = 84$) also an excellent prognostic accuracy of the CRTA in predicting mortality. Most recently, Amodeo et al. (15) also reported a good prognostic accuracy of the CRTA concerning survival. In contrast to results quoted before, Dimitriou et al. only found a weak performance of the CRTA in the prediction of poor outcome (11). In their study, only the postoperatively measured CRTA was associated with poor outcome and not—as used in the present study—the preoperatively quantified CRTA (11). One explanation can be the

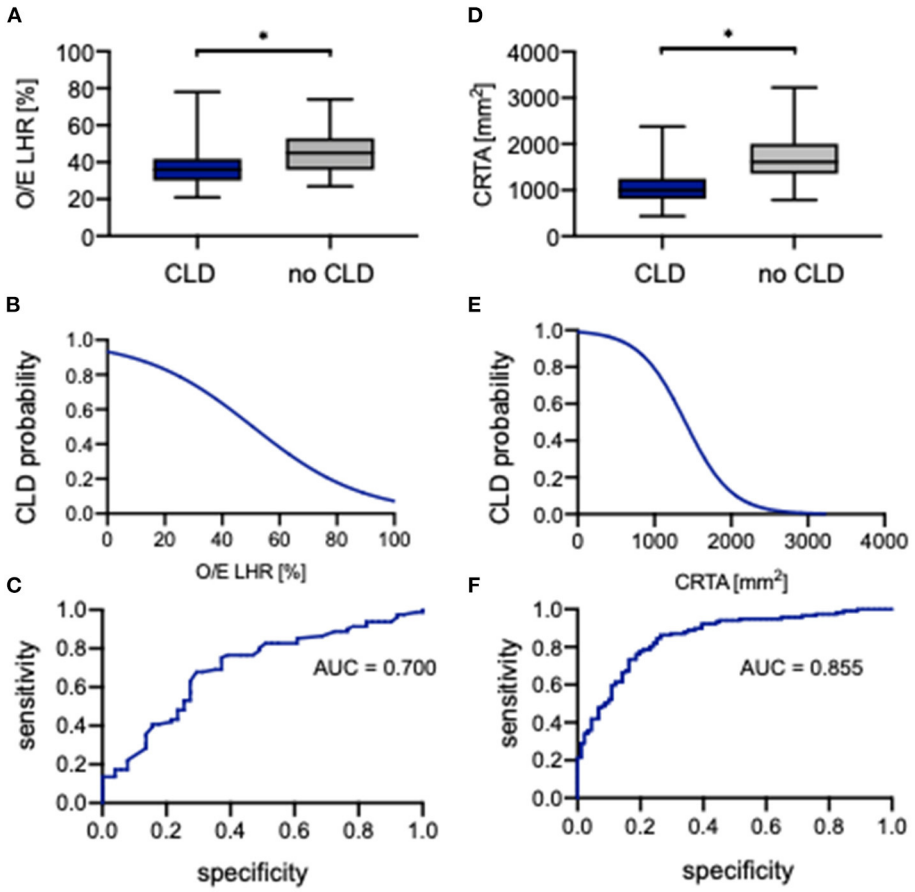


FIGURE 6 | Correlation of observed-to-expected lung-to-head ratio and CRTA to CLD. Boxplots of **(A)** observed-to-expected lung-to-head ratio and **(D)** CRTA are exhibited for patients with CLD and without CLD (whiskers are shown as minimum and maximum). Also shown is the logistical regression **(B,E)** and ROC curve **(C,F)** of observed-to-expected lung-to-head ratio and CRTA for the prediction of CLD probability in the study population. *represents a significant difference in the *U* test. AUC, area under curve; CLD, chronic lung disease; CRTA, chest radiographic thoracic area; LHR, lung-to-head ratio; O/E, observed-to-expected.

relatively small ($n = 25$) study cohort, in which also premature neonates (<34 gestational weeks) were included.

To our knowledge, there are no other studies evaluating the prognostic accuracy of the CRTA regarding ECMO requirement. Our study demonstrates an excellent prognostic accuracy for this endpoint. As ECMO therapy is available only in specialized centers, the postnatally measured CRTA can help to assess whether an outborn child should be transported into a tertiary care center.

To date, there have been few studies on the value of postnatal parameters in predicting the development of CLD in neonates with CDH. In the work of Dimitriou et al. (11), there was no correlation between preoperative CRTA and the development of CLD in CDH. A recently published study by Amodeo et al. (15) showed a significant correlation between CRTA and long-term lung function morbidity in the follow-up of neonates with CDH.

In our investigations, CRTA values ranged from 254 to 3,225 mm^2. Dassios et al. (12) reported CRTA values between 1,000 and 2,000 mm^2 in their collective, and Dimitriou et al. (11) published values from 630 to 1,860 mm^2. Thus, the values reported are in a similar range. However, it is noticeable that the maximum and minimum values diverge more strongly in our results. Compared with the other studies, a broader spectrum of severity of CDH seems to be represented in our patient cohort.

We additionally defined cutoff values of CRTA related to the three prognostic endpoints. As far as we know, Dassios et al. (12) were the only ones who also defined cutoff values for CRTA, which was 1,299 mm^2 for survival. It is noticeable that the value for mortality in the current study is lower with 806 mm^2. A possible explanation for this could be the more heterogeneous patient population in our study. The difference between the cutoff values indicates that further investigation in a larger and multicentric patient collective is necessary before a clinical application of the cutoff values can take place.

The CRTA seems to have an increased prognostic accuracy compared with the prenatally measured and well-established O/E LHR in our study cohort when comparing the AUC values ($AUC_{survival}$ 0.822 vs. 0.674, AUC_{ECMO} 0.802 vs. 0.678, AUC_{CLD} 0.855 vs. 0.700). The value of prognostic accuracy of the O/E LHR differs between studies. For example, for survival, AUC values

from 0.732 to 0.782 have been reported from us and others (9, 16–18). For ECMO therapy, we have earlier demonstrated an AUC of 0.612, and for CLD, 0.706 (9).

One limitation of the O/E LHR is the decreased reliability in children with CDH (17). In addition, the ipsilateral lung is not included in measurements. Although it is known that measured O/E LHR correlates with magnetic resonance imaging (MRI) tomographically measured lung volume, the differences are particularly determined by different sized ipsilateral lung volumes (19). This potential underestimation of lung volume via the O/E LHR is even strengthened by the fact that the contralateral lung is more strongly compromised laterally than coronally. Therefore, apical and basal lung volume can be underrepresented by the O/E LHR (20). With prenatal MRI and measured rLV, these limitations can be overcome, and excellent prognostic accuracy for survival (AUC = 0.775), ECMO requirement (AUC = 0.741), and CLD (AUC = 0.792) has been reported. Despite these known limitations of the O/E LHR, it is a broadly used and well-established ultrasound parameter, which is why we chose it as comparison for prognostic accuracy to the CRTA.

As the O/E LHR is determined prenatally and the CRTA on day 1 postnatally, both parameters should not be regarded as competing but as complementary. The CRTA can be measured easily and quickly (approximately 1-min duration) for each child with CDH. Especially for neonates with unknown diagnosis of CDH prenatally, the CRTA seems to be a helpful tool in the prognostic assessment.

One limitation of our study is the monocentric approach of data collection. Future work will have to evaluate whether calculated cutoff values can be transferred to other centers. Another weakness of the present study is that only children with isolated congenital diaphragmatic have been included into analysis to avoid a bias, and therefore, the CRTA has not been evaluated for children with multiple malformations. Future studies should evaluate whether the CRTA is also of prognostic value in this cohort. In addition, in order to recruit a representative and large study cohort, the observation period was quite long (2013–2019). A change in postnatal management, as introduced 2015 (3), might potentially have influenced the outcome parameters and, consequently, the cutoff values of analysis.

CONCLUSIONS

The CRTA can be easily measured in the postnatally acquired chest x-ray for each child with CDH. Despite its simplicity, it shows an excellent prognostic accuracy for important outcome parameters and should therefore be additionally used in the prognostic assessment of children with CDH.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee II of the Medical Faculty Mannheim of the University of Heidelberg (reference number: 2020-802R). Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MW, SB, TS, and NR contributed to the concept and design, acquisition, interpretation of data, and drafting of the article. AP, ON, SH, KZ, and SS contributed to the interpretation of data and revised the article for important intellectual content. All authors approved the final version of the article.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.740941/full#supplementary-material

REFERENCES

1. Rafat N, Schaible T. Extracorporeal membrane oxygenation in congenital diaphragmatic hernia. *Front Pediatr.* (2019) 7:336. doi: 10.3389/fped.2019.00336
2. Baird R, MacNab YC, Skarsgard ED. Mortality prediction in congenital diaphragmatic hernia. *J Pediatr Surg.* (2008) 43:783–7. doi: 10.1016/j.jpedsurg.2007.12.012
3. Snoek KG, Reiss IKM, Greenough A, Capolupo I, Urlesberger B, Wessel L, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH EURO consortium consensus - 2015 update. *Neonatology.* (2016) 110:66–74. doi: 10.1159/000444210
4. Russo FM, Cordier AG, De Catte L, Saada J, Benachi A, Deprest J. Proposal for standardized prenatal ultrasound assessment of the fetus with congenital diaphragmatic hernia by the European reference network on rare inherited and congenital anomalies (ERNICA). *Prenat Diagn.* (2018) 38:629–37. doi: 10.1002/pd.5297
5. Cordier AG, Russo FM, Deprest J, Benachi A. Prenatal diagnosis, imaging, and prognosis in congenital diaphragmatic hernia. *Semin Perinatol.* (2020) 44:51163. doi: 10.1053/j.semperi.2019.07.002
6. Cordier AG, Cannie MM, Guilbaud L, De Laveaucoupet J, Martinovic J, Nowakowska D, et al. Stomach position versus liver-to-thoracic volume ratio in left-sided congenital diaphragmatic hernia. *J Matern Neonatal Med.* (2015) 28:190–5. doi: 10.3109/14767058.2014.906576
7. Kehl S, Siemer J, Brunnemer S, Weiss C, Eckert S, Schaible T, et al. Prediction of postnatal outcomes in fetuses with isolated congenital diaphragmatic hernias using different lung-to-head ratio measurements. *J Ultrasound Med.* (2014) 33:759–67. doi: 10.7863/ultra.33.5.759
8. Alfaraj MA, Shah PS, Bohn D, Pantazi S, O'Brien K, Chiu PP, et al. Congenital diaphragmatic hernia: Lung-to-head ratio and lung volume

for prediction of outcome. *Am J Obstet Gynecol*. (2011) 205:43.e1–43.e8. doi: 10.1016/j.ajog.2011.02.050
9. Kastenholz KE, Weis M, Hagelstein C, Weiss C, Kehl S, Schaible T, et al. Correlation of observed-to-expected MRI fetal lung volume and ultrasound lung-to-head ratio at different gestational times in fetuses with congenital diaphragmatic hernia. *Am J Roentgenol.* (2016) 206:856–66. doi: 10.2214/AJR.15.15018
10. Dimitriou G, Greenough A, Kavvadia V, Shute M, Karani J. A radiographic method for assessing lung area in neonates. *Br J Radiol.* (1999) 72:335–8. doi: 10.1259/bjr.72.856.10474492
11. Dimitriou G, Greenough A, Davenport M, Nicolaides K. Prediction of outcome by computer-assisted analysis of lung area on the chest radiograph of infants with congenital diaphragmatic hernia. *J Pediatr Surg.* (2000) 35:489–93. doi: 10.1016/S0022-3468(00)90219-7
12. Dassios T, Ali K, Makin E, Bhat R, Krokidis M, Greenough A. Prediction of mortality in newborn infants with severe congenital diaphragmatic hernia using the chest radiographic thoracic area. *Pediatr Crit Care Med.* (2019) 20:534–9. doi: 10.1097/PCC.0000000000001912
13. Schaible T, Büsing KA, Felix JF, Hop WCJ, Zahn K, Wessel L, et al. Prediction of chronic lung disease, survival and need for ECMO therapy in infants with congenital diaphragmatic hernia: additional value of fetal MRI measurements? *Eur J Radiol.* (2012) 81:1076–82. doi: 10.1016/j.ejrad.2011.02.060
14. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *Am J Respir Crit Care Med.* (2001) 163:1723–9. doi: 10.1164/ajrccm.163.7.2011060
15. Amodeo I, Raffaeli G, Pesenti N, Macchini F, Condò V, Borzani I, et al. The NeoAPACHE study protocol i: assessment of the radiographic pulmonary area and long-term respiratory function in newborns with congenital diaphragmatic hernia. *Front Pediatr.* (2020) 8:581809. doi: 10.3389/fped.2020.581809
16. Jani J, Cannie M, Sonigo P, Robert Y, Moreno O, Benachi A, et al. Value of prenatal magnetic resonance imaging in the prediction of postnatal outcome in fetuses with diaphragmatic hernia. *Ultrasound Obstet Gynecol.* (2008) 32:793–9. doi: 10.1002/uog.6234
17. Jani J, Peralta CFA, Benachi A, Deprest J, Nicolaides KH. Assessment of lung area in fetuses with congenital diaphragmatic hernia. *Ultrasound Obstet Gynecol.* (2007) 30:72–6. doi: 10.1002/uog.4051
18. Jani JC, Peralta CFA, Ruano R, Benachi A, Done E, Nicolaides KH, et al. Comparison of fetal lung area to head circumference ratio with lung volume in the prediction of postnatal outcome in diaphragmatic hernia. *Ultrasound Obstet Gynecol.* (2007) 30:850–4. doi: 10.1002/uog.5167
19. Jani J, Cannie M, Done E, Van Mieghem T, Van Schoubroeck D, Gucciardo L, et al. Relationship between lung area at ultrasound examination and lung volume assessment with magnetic resonance imaging in isolated congenital diaphragmatic hernia. *Ultrasound Obstet Gynecol.* (2007) 30:855–60. doi: 10.1002/uog.5168
20. Jani J, Peralta CFA, Van Schoubroeck D, Deprest J, Nicolaides KH. Relationship between lung-to-head ratio and lung volume in normal fetuses and fetuses with diaphragmatic hernia. *Ultrasound Obstet Gynecol.* (2006) 27:545–50. doi: 10.1002/uog.2735

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MINI REVIEW
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Respiratory Support of Infants With Congenital Diaphragmatic Hernia

Emma Williams[1] *and Anne Greenough*[1,2,3]*

[1] Department of Women and Children's Health, School of Life Course Sciences, Faculty of Life Sciences and Medicine, King's College London, London, United Kingdom, [2] Asthma UK Centre for Allergic Mechanisms in Asthma, King's College London, London, United Kingdom, [3] National Institute for Health Research (NIHR) Biomedical Research Centre at Guy's and St Thomas' National Health Service (NHS) Foundation Trust and King's College London, London, United Kingdom

Optimisation of respiratory support of infants with congenital diaphragmatic hernia (CDH) is critical. Infants with CDH often have severe lung hypoplasia and abnormal development of their pulmonary vasculature, leading to ventilation perfusion mismatch. It is vital that lung protective ventilation strategies are employed during both initial stabilisation and post-surgical repair to avoid ventilator induced lung damage and oxygen toxicity to prevent further impairment to an already diminished gas-exchanging environment. There is a lack of robust evidence for the routine use of surfactant therapy during initial resuscitation of infants with CDH and thus administration cannot be recommended outside clinical trials. Additionally, inhaled nitric oxide has been shown to have no benefit in reducing the mortality rates of infants with CDH. Other therapeutic agents which beneficially act on pulmonary hypertension are currently being assessed in infants with CDH in randomised multicentre trials. The role of novel ventilatory modalities such as closed loop automated oxygen control, liquid ventilation and heliox therapy may offer promise for infants with CDH, but the benefits need to be determined in appropriately designed clinical trials.

Keywords: mechanical ventilation, pressure controlled ventilation, volume controlled ventilation, high frequency oscillation, surfactant, inhaled nitric oxide

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Martin Keszler, Women & Infants Hospital of Rhode Island, United States

***Correspondence:**
Anne Greenough
anne.greenough@kcl.ac.uk

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INTRODUCTION

The developmental disruption to the lungs and pulmonary vasculature of newborn infants with congenital diaphragmatic hernia (CDH) poses challenges during adaptation to postnatal life. The function of the lungs in providing essential gas exchange of oxygen and carbon dioxide at the alveolar-capillary membrane can be greatly diminished in infants presenting with this congenital abnormality (1). Importantly, appropriate, and timely intervention by clinicians is necessary to provide what can often be life-saving treatment, but may further adversely affect gas exchange. Ventilatory strategies and modalities are continuously being developed, with advances in technology contributing to such techniques. This review aims to provide clinicians with an outline of the recent evidence based ventilatory options available from birth and the initial stabilisation, through to surgery and post-operative management. It will also provide an insight into future developments.

VENTILATORY MANAGEMENT DURING RESUSCITATION

Guidelines from the European Congenital Diaphragmatic Hernia (CDH EURO) Consortium and the American Academy of Pediatrics and American Heart Association (AAP/AHA) recommend routine intubation at birth of all infants with CDH where the prenatal diagnosis is known, with avoidance of bag and mask ventilation and the resultant inflation of herniated bowel contents (2). Peak inspiratory pressures of <25 cmH_2O are recommended to avoid ventilator induced lung injury (VILI) to both lungs (2).

Respiratory function monitoring can measure the response to initial resuscitation and the results utilised to predict subsequent survival. Expiratory tidal volumes ($p = 0.009$) and lung compliance ($p = 0.03$) measured during the first minute of recorded resuscitation have been shown to be lower in non-survivors. An expiratory tidal volume of >3.8 ml/kg and a lung compliance of >0.12 ml/cmH_20/kg was reported to be predictive of survival with 85% sensitivity and 90% specificity. Furthermore, tidal volumes of spontaneous breaths measured during the first 10 min after intubation have been described to be lower in those in those who either died prior to discharge or in those who developed chronic lung disease compared to survival without chronic lung disease (2.0 vs. 4.3 ml/kg; $p = 0.004$) (3). The achievement of higher maximal pre-ductal oxygen saturations (100 vs. 93%; $p = 0.037$) prior to transfer to neonatal intensive care have also been associated with greater survival in infants with a diagnosis of CDH (4). Such results are reflective of the degree of pulmonary hypoplasia in non-survivors. Additionally, respiratory function monitoring can be utilised to calculate the anatomical dead space in those with congenital malformations affecting the lungs. A larger anatomical dead space has been reported in those infants with CDH who survived to discharge [2.9 (2.8–3.3) ml/kg] compared to those who died [2.2 (2.1–2.7) ml/kg; $p = 0.003$] and can be used to predict survival [area under the curve (AUC) = 0.90] (5).

Dynamic lung compliance is low at birth and has been shown to be adversely affected by administration of a neuromuscular blocking agent. Indeed, the median lung compliance in a cohort of 15 infants with antenatally diagnosed CDH reduced from 0.22 to 0.16 ml.cmH_2O^{-1}.kg^{-1} ($p < 0.001$) immediately post administration of pancuronium bromide (6) (**Figure 1**). Hence, neuromuscular blocking agents should not be routinely administered during resuscitation (**Figure 1**). The respiratory function monitor used for pulmonary assessment within this study did not, however, measure oesophageal pressure

During resuscitation, physiological based cord clamping in animal models has been shown to be beneficial as dilatation of the pulmonary vasculature occurs following lung aeration, and thus pulmonary blood flow increases and oxygenation is improved (7). The feasibility of intact cord resuscitation in infants with CDH has recently been assessed in pilot studies. One prospective observational study ($n = 40$) reported no increase in neonatal adverse events whilst initiating resuscitation prior to cord clamping (8). A single-arm safety study ($n = 20$) determined the feasibility of intubation and ventilation prior to cord clamping and subsequently reported no significant difference in oxygenation indices or need for subsequent vasoactive therapy compared to those CDH infants undergoing immediate umbilical cord clamping (9). Recruitment is in progress into a multicentre international randomised trial determining the impact of physiological based cord clamping on clinically relevant outcomes in infants with CDH (10).

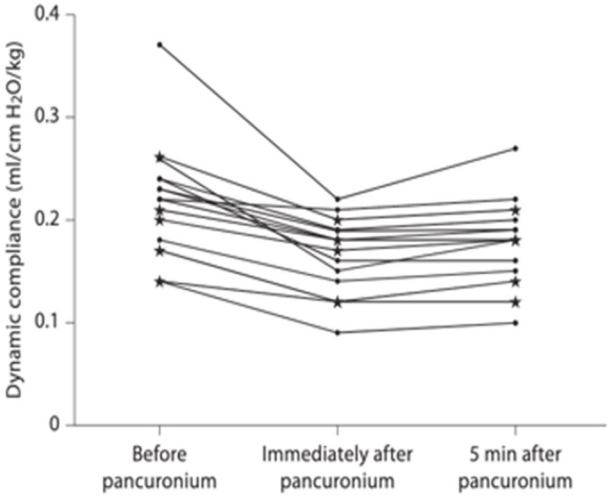

FIGURE 1 | Dynamic compliance immediately before, immediately after and 5 min after pancuronium bromide administration [taken from (6)].

Where adequate antenatal development of the lung is expected, spontaneous breathing at birth can be considered. A recent, retrospective cohort study of 18 infants with mild CDH found a spontaneous breathing approach at delivery to be both safe and feasible (11). Prospective randomised trials are needed to appropriately evaluate this approach.

INITIAL VENTILATORY MANAGEMENT PRE-SURGERY

Determining the optimal initial mode of ventilation in infants with CDH was assessed in a randomised trial (the VICI-trial) (12). Lung volume recruitment strategies utilised by high frequency oscillatory ventilation (HFOV) were not found to be superior to conventional mechanical ventilation (CMV) in reducing the combined outcome of death or bronchopulmonary dysplasia (BPD) [OR 0.62 (95% CI 0.25–1.55), $p = 0.31$]. With regards to secondary outcomes, however, the median (IQR) duration of mechanical ventilation was lower in the CMV group [10 (6–18) days] than the HFOV group [13 (8–23) days, $p = 0.03$]. Given the underlying lung pathology in CDH is that of pulmonary hypoplasia (a non-recruitable lung disease) this likely explains the inferiority of HFOV compared to CMV. Of those randomised to CMV, 42.9% required treatment with inhaled nitric oxide, compared to 56.2% of those on HFOV ($p = 0.045$). Additionally, a greater duration of vasoactive medication was needed in the HFOV [8 (4.3–19) days] compared to the CMV

group [6 (3.3–11.8) days, $p = 0.02$]. The VICI trial also showed conventional ventilatory support to be beneficial in reducing the requirement for extracorporeal membrane oxygenation support (ECMO) (26 vs. 51%, $p = 0.007$) and is thus suggested as first line choice of ventilatory support in infants with CDH (2, 13, 14). Such benefits of CMV seen with regards to the secondary outcomes may however be reflective of the higher starting mean airway pressure (MAP) in the HFOV group (initial MAP 13–17 cmH_20). A recent, multicentre cohort study of 328 infants also demonstrated no significant differences between HFOV or CMV as initial mode of ventilation when reporting on the outcome of mortality [OR 0.98 (95% CI 0.57–1.67)] or BPD [OR 1.66 (95% CI 0.50–5.49)] (15). That study, however, is limited by its retrospective nature, although propensity score matching was performed to reduce potential confounding. Furthermore, no significant difference was reported between mode of respiratory support (HFOV or CMV) at the time of surgical repair when considering oxygen dependency or death at 28 days (16). Use of high positive end-expiratory pressure (PEEP) may cause lung injury by over-inflation of the ipsilateral lung during HFOV and the ensuing pulmonary inflammatory response (12, 17). Nevertheless, use of HFOV may be indicated as rescue therapy following failure of initial conventional ventilatory strategies (18). Failure of conventional ventilation in CDH infants is considered when peak inspiratory pressures higher than 28 cmH_2O are required to maintain oxygen saturations within target range and the partial pressure of carbon dioxide (pCO_2) between 50 and 70 mmHg (2) (**Figure 2**).

LUNG PROTECTIVE VENTILATORY STRATEGIES

Pressure-Controlled Ventilation and Permissive Hypercapnia

Pressure controlled ventilation and permissive hypercapnia are strategies employed to avoid damage to the lung contralateral to the herniation. Indeed, a reduction in mortality was demonstrated in infants with CDH when pCO_2 levels as high as 70 mmHg were permitted (42.9 vs. 14.3%; $p = 0.002$) (19). That study, however, only reported outcomes at a single institution before and after the introduction of permissive hypercapnia as a therapeutic strategy over a 16-year period. The results may, therefore, have been influenced by other significant changes and advances in management during that time. To avoid ventilatory induced lung damage, low ventilatory pressures are advised by the CDH EURO Consortium, with peak inspiratory pressures of <25 cmH_2O recommended for use if possible, however that upper limit is not evidence based (2, 20).

Volume-Targeted Ventilation

The tidal volumes required to maintain effective minute ventilation and clearance of carbon dioxide in infants with CDH have been reported to be similar to that of control infants both pre (4.7 vs. 4.9 ml/kg; $p = 0.49$) and post (4.5 vs. 4.9 ml/kg; $p = 0.14$) operatively, however, in that retrospective cohort study, episodes in which tidal volumes corresponded with hypercapnic episodes were excluded. The most appropriate tidal volume targets in infants with CDH remains unanswered. Indeed, too low tidal volumes will result in an increase in the dead space to tidal volume ratio, yet use of too high tidal volumes may over-distend the already fewer alveoli existing in hypoplastic CDH lungs (20). No randomised trials have yet been performed to determine the optimal tidal volumes targets in infants with pulmonary hypoplasia secondary to congenital diaphragmatic hernia (21).

INTRA-OPERATIVE VENTILATION

A pilot, randomised controlled trial aimed to determine the effect of open or thoracoscopic repair on intraoperative acidosis and hypercapnia. The thoracoscopic approach was associated with higher levels of intraoperative partial pressure of carbon dioxide (pCO_2) (83 vs. 61 mmHg; $p = 0.036$) and prolonged acidosis compared to open CDH repair (14). The insufflation of CO_2 during thoracoscopic repair was thought to adversely impact upon intra-operative ventilation; all the infants were supported by conventional ventilation. Subsequent potential countermeasures to hypercapnia during thoracoscopic repair, such as intrapulmonary percussive ventilation and pausing the insufflation of CO_2 during surgery, have resulted in non-significant differences in the maximal pCO_2 levels during thoracoscopic [55.9 (38–192) mmHg] and laparoscopic repair [54.1 (41–72) mmHg] ($p = 0.60$) (22). An observational study reported fewer infants with CDH to have hypercapnic (>60 mmHg) or hypoxic ($SpO_2 < 90\%$) episodes during thoracoscopic assisted repair than open repair, with a shorter duration of post-operative mechanical ventilation in the former group ($p < 0.05$) (23), but there was no reported significant difference in the overall post-operative survival rates between either choice of surgical repair.

Retrospective cohort analysis of infants who underwent thoracoscopic repair reported an increase in intraoperative pCO_2, with subsequent acidosis regardless of whether conventional or high frequency ventilation was used. Intra-operative pCO_2 was not significantly different when HFOV or CMV were chosen as the ventilatory modality during surgery; however, the infants receiving HFOV during thoracoscopic repair exhibited less marked respiratory acidosis compared to their pre-surgery values than infants supported by conventional ventilation (24). These results suggest that utilisation of HFOV during thoracoscopic repair may prevent deterioration of respiratory acidosis to a larger degree than conventional ventilation.

VENTILATORY MANAGEMENT POST-SURGICAL REPAIR

Respiratory compliance has been reported to decrease in infants with CDH following surgical repair (25). Furthermore, weight corrected respiratory system compliance (Crs) measured following surgery, in those infants with left sided CDH, has been shown to be negatively correlated with the need for prolonged post-operative mechanical ventilation ($p = 0.006$) (26). Positive end-expiratory pressure (pEEP) levels following surgical repair can affect respiratory system compliance and

FIGURE 2 | Flow diagram of initial ventilatory strategies.

resistance in those with mild-moderate CDH and persistent pulmonary hypertension. In a randomised 1-h crossover trial, lung compliance increased by 30% when PEEP levels of 2 cmH_2O were applied in comparison to use of 5 cmH_2O of PEEP ($p = 0.0001$) (27). The improvement in oxygenation that occurred at the lower level of PEEP of 2 cmH_2O, suggests higher PEEP levels are associated with over-distension of aerated "open" alveoli primarily within the ipsilateral lung (27). That study, however, was performed after surgical repair of the defect and so it remains to be answered whether low PEEP levels are beneficial pre-repair of the defect, given that the presence of viscera within the chest cavity may indeed prevent over-distension of the ipsilateral lung. Furthermore, high PEEP levels immediately after birth have been shown in an animal model to be beneficial in establishing functional residual capacity (28).

Prone positioning of mechanically ventilated infants post-operative repair of CDH has been associated with an improvement in oxygenation (PaO_2/FiO_2 ratio) ($p = 0.032$) and a reduction in the alveolar-arterial oxygen difference ($p = 0.043$) (29). Limitations of that study, however, were that measures of oxygenation and respiratory function were only measured for 30 min in each position and were not related to the adverse longer-term pulmonary outcomes suffered by infants with CDH. Post-surgical ventilation with tidal volumes of <5 ml/kg have been associated with an increase in the work of breathing in infants with CDH ($p = 0.001$) (30). That study, however, only included seven infants and future randomised, adequately powered studies are necessary to determine optimal tidal volume levels pre and post repair. Furthermore, the impact of such ventilatory strategies in relation to long-term pulmonary outcomes needs to be ascertained.

ADDITIONAL THERAPIES

Supplementary Oxygen

There is a lack of randomised control trials determining the optimal fraction of supplemental oxygen during resuscitation in infants with CDH. In view of such paucity of robust data it is speculated that a starting fraction of the inspired oxygen (FiO_2) of <1.0 during the initial resuscitation of newborn infants with CDH may be beneficial, with subsequent titration of the FiO_2 to maintain preductal peripheral oxygen saturations (SpO_2) between 80 and 95% (2). This starting FiO_2 comes as a consequence of increasing concern related to the unfavourable effects of oxidative stress (31). Reducing free radical formation by lowering the levels of the inspired oxygen may subsequently reduce pulmonary vasoconstriction and the associated adverse consequences (32–34). Furthermore, animal models of persistent pulmonary hypertension of the newborn (PPHN) have shown that resuscitation with high levels of inspired oxygen (FiO_2 1.0 vs. 0.5) can also impair the later pulmonary vasodilator response to inhaled nitric oxygen (iNO) which may be required as rescue therapy for respiratory failure in infants with CDH (35, 36). A lower starting FiO_2 (0.5) during the resuscitation of infants with CDH has been shown to have no adverse effects upon survival (adjusted $p = 0.142$) or need for ECMO (adjusted $p = 0.159$) than starting resuscitation at a higher FiO_2 (1.0) (37). If the SpO_2, however, remained low and there was a subsequent need to increase the FiO_2 to 1.0, then this was associated with a trend in reduced survival rates and postnatal ECMO requirement, but the worse outcomes were no longer significant after controlling for a lower gestational age at birth, liver position and lung-head ratio (LHR) (37). One limitation of those results is the retrospective cohort nature of the study. Future trials that randomise newborn infants with CDH to different starting levels of FiO_2 during resuscitation are needed to provide clinicians with evidence based FiO_2 targets and the later relationship to postnatal outcomes.

Knowledge of the partial pressure of arterial oxygen (PaO_2) values are necessary to guide oxygenation indices and determine criteria for ECMO, with continuous monitoring of SpO_2 utilised to guide optimal ventilatory strategies. The aim stated by consensus guidelines, is to achieve preductal oxygen saturations of between 80 and 95% 2 h after birth, with post ductal saturations above 70% (2). Provision of supplemental oxygen titrated to such SpO_2 levels does, however, need careful monitoring (38, 39). Animal models of the CDH ventilatory responses during the first 2 h after birth have recently demonstrated unintentional cerebral hyperoxia to occur when cerebral blood flow is unmonitored, thought to be related to the rapid increases in carotid blood flow (38). Cerebral oxygenation, monitored by near-infrared spectroscopy, has been reported to be reduced during surgical repair of infants with severe CDH, regardless of conventional or high frequency modes of ventilatory support ($p = 0.0001$) (39). Those infants receiving HFOV however exhibited a prolonged reduction in cerebral oxygenation and required a longer duration of time to recover to normal values than

those in the conventional group ($p = 0.003$) (39). The study, however, was not appropriately powered to determine the effects of mode of ventilation on cerebral oxygenation during surgery. Furthermore, the relationship of such findings with longer term neurodevelopmental outcomes were not reported.

Use of Inhaled Nitric Oxide

Inhaled nitric oxide (iNO) use in newborn infants with CDH has been reported in 68 (97.1%) participating centres in the Congenital Diaphragmatic Hernia Study Group registry (40). Of 2,174 infants diagnosed by echocardiography with pulmonary hypertension (PH) 74.2% received iNO therapy, however 36.4% of infants without PH were also treated with iNO. Propensity score analysis revealed iNO use to be associated with a 15% higher absolute mortality rate (average treatment effect on the treated: 0.15; 95% CI 0.10–0.20) (40). The use of iNO therapy for CDH infants with hypoxemic respiratory failure, unresponsive to conventional therapy, has also not been found to decrease the need for ECMO or reduce the primary outcome of death before discharge (41). Furthermore, only 16% of infants in that study (41) fully responded to iNO therapy at a dosage of 20 parts per million (ppm), as determined by an improvement of oxygenation indices. Inhaled nitric oxide is, however, often the first line drug of choice for pulmonary hypertension in newborn infants with CDH and forms a standard of care for the CDH Euro Consortium group (2). Nevertheless, as iNO has not been shown to be beneficial in reducing mortality rates, it is not routinely recommended by the American Pediatric Surgical Association (APSA) for the treatment of pulmonary hypertension in infants with CDH (13). Further trials are underway to determine if other therapeutic agents which act to reduce PH may offer more promise in infants with CDH. Currently, an international multicentre, randomised controlled trial is in the recruitment phase to assess whether intravenous sildenafil may be superior to iNO in reducing mortality in newborns with CDH (42). Additional trials are also setting out to determine the beneficial effects of inotropic agents, such as milrinone, in treating pulmonary hypertension caused by right and left ventricular dysfunction in CDH. Such therapy may improve left ventricular diastolic and systolic function, reduce afterload and subsequently lead to improved oxygenation in those with CDH. A randomised pilot trial is being conducted to determine the safety and feasibility of undertaking a larger multicentre trial (43).

Surfactant Therapy

Infants with CDH often exhibit reduced lung compliance (44). The use, therefore, of postnatal surfactant has been considered in infants with CDH. Use of postnatal surfactant administered to term infants (>37 weeks' gestational age, $n = 522$) was reported to have no beneficial impact upon survival, nor did such treatment reduce the incidence of chronic lung disease or the need for extra-corporeal membrane oxygenation (ECMO) (45). Furthermore, retrospective data from the CDH registry reported that administration of surfactant to preterm infants (<37 weeks' gestation, $n = 424$) was associated with a greater risk of death before discharge [odds ratio (OR) 2.17, 95% CI: 1.5–3.2; $p < 0.01$] (46). Additionally, surfactant replacement given to infants >35 weeks of gestation whilst on ECMO was reported to have no beneficial effects on the outcomes of survival to discharge (OR 1.0, 95% CI 0.67–1.62; $p = 0.87$) or requirement for supplemental home oxygen (OR 1.04, 95% CI 0.6–1.8; $p = 0.90$) (47). Surfactant concentrations in human foetuses with CDH have been reported to be similar to those of age matched controls, moreover the maturation and storage of surfactant appears not adversely impacted by this congenital pulmonary abnormality (48). Routine surfactant administration to infants with CDH is therefore not currently recommended in the EURO consensus guidance (2). Randomised clinical trials (RCTs) for surfactant therapy in preterm infants with CDH are warranted. If postnatal surfactant is given it should be noted that the standardised dosage regimens for weight are likely to be inaccurate due to the degree of pulmonary hypoplasia (48).

NOVEL VENTILATORY MODALITIES

Neurally Adjusted Ventilatory Assist

Neurally adjusted ventilatory assist (NAVA) may confer protection to hypoplastic lungs. During NAVA, ventilatory support is delivered in response to diaphragmatic electrical activity. The utility of NAVA in newborn infants with structural diaphragmatic abnormalities has not been widely studied and since this ventilatory mode is dependent upon detection of neural diaphragmatic signals certain challenges may arise. In one study, after primary repair, infants with CDH placed on invasive NAVA were shown to have no differences in peak or resting electrical activity of the diaphragm during respiration compared to control infants with no underlying diaphragmatic abnormality, nor did they require higher levels of NAVA support (49). A recent case control study ($n = 16$) found no significant difference in the NAVA level ($p = 0.286$) used post-surgical repair in infants with CDH compared to those without CDH of similar age and weight at the time of study. Furthermore, NAVA use in those with diaphragmatic hernia was associated with a reduction in ventilatory requirements and the need for sedative therapy (49). Two retrospective feasibility studies performed in infants with CDH following surgical repair showed that post-operative weaning of ventilation with both invasive ($n = 10$) and non-invasive ($n = 7$) NAVA to be successful (50, 51). Additionally, the short-term outcomes of infants with CDH who have been placed on invasive NAVA post-surgery were assessed and NAVA was associated with a decrease in mean airway pressure ($p < 0.001$) and respiratory severity score ($p < 0.001$) at 72 h post initiation ($p < 0.001$) (49).

Closed Loop Automated Oxygen Control

Closed loop automated oxygen control systems have yet to be trialled in infants with CDH, but recent developments have shown promising results in optimising ventilatory support in other pulmonary conditions (52–56). Whether such a modality has a role in infants with congenital diaphragmatic anomalies has yet to be determined.

Heliox Therapy

Utilising heliox as an adjunctive therapy to the ventilation of infants with CDH was shown in one retrospective cohort study to be beneficial in reducing levels of hypercapnia (68 vs. 49 mmHg; $p < 0.001$) and levels of maximal ventilatory support required from high frequency oscillatory ventilation, and thus may be one such therapy to improve gas exchange in those with lung hypoplasia (57). Prospective randomised trials are required to ascertain the benefit of such therapy on short- and long-term pulmonary outcomes.

Liquid Ventilation

Lung growth *in utero* occurs as pulmonary fluid secretion provides a continuous distending pressure to the airways (58). Fluid filled lungs, combined with foetal breathing movements, underlie the mechanisms behind antenatal lung growth (59). Liquid at room temperature and less viscous that water, perfluorocarbons may thus provide some benefit when conventional ventilatory strategies remain challenging during postnatal life (60). By providing constant distending pressure, liquid ventilation may improve lung mechanics in a similar fashion to that of an increased PEEP. Results from animal models of severe respiratory failure have indeed shown partial liquid ventilation to be beneficial with regard to gas exchange and reducing pulmonary shunting (88 vs. 31%; $p < 0.001$) during ECMO (61). A randomised trial to assess the feasibility of partial liquid ventilation (perfluorocarbon-induced lung growth) in newborn infants with CDH ($n = 13$) confirmed the short-term safety of performance of this novel technique during ECMO (62). Larger randomised trials would be required to ascertain the long-term safety and benefits of liquid ventilation as a postnatal strategy in infants born with congenital diaphragmatic hernia, however as obtaining regulatory approval may prove challenging such therapy may be of doubtful clinical relevance.

LONG TERM LUNG FUNCTION

Pulmonary morbidity and long-term lung function of infants with CDH is now of more significance as survival rates have increased (63). Follow up of 28 children with repaired left sided CDH (mean age 6.2 years) revealed 25% had abnormal pulmonary function ($p < 0.01$). Furthermore, those with abnormal pulmonary function had lower total lung volume on structural evaluation of chest tomography (826.5 $\pm$ 133.6 mls) than those with normal overall lung function (1,244.5 $\pm$ 407.9 mls; $p < 0.05$) (64). Longitudinal analysis of ventilation-perfusion (V/Q) mismatching has been recently reported in CDH survivors (65). In those with severe disease ipsilateral V/Q mismatch worsenend over time likely due to the progressive reduction in pulmonary perfusion ($p = 0.012$). Such perfusion deficits may be related to abnormal lung function and V/Q studies may thus be an important addition to exercise testing in order to identify and monitor those most at risk of worse outcomes.

CONCLUSION

This review has highlighted respiratory management techniques which could be utilised in resusciation and during the pre and post operative management stages in infants with CDH. Respiratory function monitoring is a useful tool to monitor pulmonary mechanics and the results may be utilised to predict subsequent survival. Conventional ventilatory modes are recommenced as initial respiratory support given the primary lung pathology of pulmonary hypoplasia. Further research and more randomised trials are, however, needed to provide clinicians with evidence based, optimal respiratory management strategies which have been shown to improve the long term pulmonary outcomes of infants with congenital diaphgramatic hernia.

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AG and EW designed the review and contributed to its production. Both authors contributed to the article and approved the submitted version.

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REFERENCES

1. Ting A, Glick PL, Wilcox DT, Holm BA, Gil J, DiMaio M. Alveolar vascularization of the lung in a lamb model of congenital diaphragmatic hernia. *Am J Respir Crit Care Med.* (1998) 157:31–4. doi: 10.1164/ajrccm.157.1.9703034
2. Snoek KG, Reiss IK, Greenough A, Capolupo I, Urlesberger B, Wessel L, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: The CDH EURO Consortium Consensus - 2015 Update. *Neonatology.* (2016) 110:66–74. doi: 10.1159/000444210
3. Mank A, Carrasco Carrasco C, Thio M, Clotet J, Pauws SC, DeKoninck P, et al. Tidal volumes at birth as predictor for adverse outcome in congenital diaphragmatic hernia. *Arch Dis Child.* (2020) 105:248–52. doi: 10.1136/archdischild-2018-316504
4. O'Rourke-Potocki A, Ali K, Murthy V, Milner A, Greenough A. Resuscitation of infants with congenital diaphragmatic hernia. *Arch Dis Child Fetal Neonatal Ed.* (2017) 102:F320–3. doi: 10.1136/archdischild-2016-311432
5. Williams EE, Dassios T, Murthy V, Greenough A. Anatomical deadspace during resuscitation of infants with congenital diaphragmatic hernia. *Early Hum Dev.* (2020) 149:105150. doi: 10.1016/j.earlhumdev.2020.105150
6. Murthy V, D'Costa W, Nicolaides K, Davenport M, Fox G, Milner AD, et al. Neuromuscular blockade and lung function during resuscitation of infants with congenital diaphragmatic hernia. *Neonatology.* (2013) 103:112–7. doi: 10.1159/000342332
7. Polglase GR, Blank DA, Barton SK, Miller SL, Stojanovska V, Kluckow M, et al. Physiologically based cord clamping stabilises cardiac output and reduces cerebrovascular injury in asphyxiated near-term lambs. *Arch Dis Child Fetal Neonatal Ed.* (2018) 103:F530–8. doi: 10.1136/archdischild-2017-313657

8. Lefebvre C, Rakza T, Weslinck N, Vaast P, Houfflin-Debarge V, Mur S, et al. Feasibility and safety of intact cord resuscitation in newborn infants with congenital diaphragmatic hernia (CDH). *Resuscitation.* (2017) 120:20–5. doi: 10.1016/j.resuscitation.2017.08.233
9. Foglia EE, Ades A, Hedrick HL, Rintoul N, Munson DA, Moldenhauer J, et al. Initiating resuscitation before umbilical cord clamping in infants with congenital diaphragmatic hernia: a pilot feasibility trial. *Arch Dis Child Fetal Neonatal Ed.* (2020) 105:322–6. doi: 10.1136/archdischild-2019-317477
10. Available online at: https://clinicaltrials.gov/ct2/show/NCT04373902.
11. Cochius-den Otter SC, Horn-Oudshoorn EJ, Allegaert K, DeKoninck PL, Peters NC, Cohen-Overbeek TE, et al. Routine intubation in newborns with congenital diaphragmatic hernia. *Pediatrics.* (2020) 146:e20201258. doi: 10.1542/peds.2020-1258
12. Snoek KG, Capolupo I, Van Rosmalen J, de Jongste vanden Hout L, Vijfhuize S, Greenough A, et al. Conventional mechanical ventilation versus high-frequency oscillatory ventilation for congenital diaphragmatic hernia. *Ann Surg.* (2016) 263:867–74. doi: 10.1097/SLA.0000000000001533
13. Puligandla PS, Grabowski J, Austin M, Hedrick H, Renaud E, Arnold M, et al. Management of congenital diaphragmatic hernia: a systematic review from the APSA outcomes and evidence based practice committee. *J PediatrSurg.* (2015) 50:1958–70. doi: 10.1016/j.jpedsurg.2015.09.010
14. Bishay M, Giacomello L, Retrosi G, Thyoka M, Garriboli M, Brierley J, et al. Hypercapnia and acidosis during open and thoracoscopic repair of congenital diaphragmatic hernia and esophageal atresia: results of a pilot randomized controlled trial. *Ann Surg.* (2013) 258:895–900. doi: 10.1097/SLA.0b013e31828fab55
15. Fuyuki M, Usui N, Taguchi T, Hayakawa M, Masumoto K, Kanamori Y, et al. Prognosis of conventional vs. high-frequency ventilation for congenital diaphragmatic hernia: a retrospective cohort study. *J Perinatol.* (2021) 41:814–23. doi: 10.1038/s41372-020-00833-6
16. Derraugh G, Levesque M, Schantz D, Sesha M, Minski J, Baier J, et al. High-frequency vs. conventional ventilation at the time of CDH repair is not associated with higher mortality and oxygen dependency: a retrospective cohort study. *Pediatr Surg Int.* (2020) 36:1275–80. doi: 10.1007/s00383-020-04740-x
17. Dreyfuss D, Saumon G. Role of tidal volume, FRC, and end-inspiratory volume in the development of pulmonary edema following mechanical ventilation. *Am Rev Respir Dis.* (1993) 148:1194–203. doi: 10.1164/ajrccm/148.5.1194
18. Attar MA, Dechert RE, Donn SM. Rescue high frequency ventilation for congenital diaphragmatic hernia. *J Neonat Perinat Med.* (2019) 12:173–8. doi: 10.3233/NPM-1813
19. Guidry CA, Hranjec T, Rodgers BM, Kane B, McGahren ED. Permissive hypercapnia in the management of congenital diaphragmatic hernia: our institutional experience. *J Am Coll Surg.* (2012) 214:640–7. doi: 10.1016/j.jamcollsurg.2011.12.036
20. Keszler M. Mechanical ventilation strategies. *Semin Fetal Neonat Med.* (2017) 22:267–74. doi: 10.1016/j.siny.2017.06.003
21. Duncan KV, Polites S, Krishnaswami S, Scottoline BP. Congenital diaphragmatic hernia management: a systematic review and care pathway description including volume-targeted ventilation. *Adv Neonat Care.* (2021) 21:E138–43. doi: 10.1097/ANC.0000000000000863
22. Inoue M, Uchida K, Otake K, Nagano Y, Mori K, Hashimoto K, et al. Thoracoscopic repair of congenital diaphragmatic hernia with countermeasures against reported complications for safe outcomes comparable to laparotomy. *Surg Endosc.* (2016) 30:1014–9. doi: 10.1007/s00464-015-4287-6
23. Qin J, Ren Y, Ma D. A comparative study of thoracoscopic and open surgery of congenital diaphragmatic hernia in neonates. *J Cardiothorac Surg.* (2019) 14:118. doi: 10.1186/s13019-019-0938-3
24. Okazaki T, Okawada M, Ishii J, Koga H, Miyano G, Doi T, et al. Intraoperative ventilation during thoracoscopic repair of neonatal congenital diaphragmatic hernia. *Pediatr Surg Int.* (2017) 33:1097–101. doi: 10.1007/s00383-017-4143-y
25. Sakai H, Tamura M, Hosokawa Y, Bryan AC, Barker GA, Bohn DJ. Effect of surgical repair on respiratory mechanics in congenital diaphragmatic hernia. *J Pediatr.* (1987) 111:432–8. doi: 10.1016/S0022-3476(87)80475-4
26. Takeuchi M, Kinouchi K, Fukumitsu K, Imura K, Kitamura S. Respiratory system compliance and postoperative ventilator dependence in neonates with left-sided congenital diaphragmatic hernia. *J Anesth.* (2001) 15:139–44. doi: 10.1007/s005400170015
27. Guevorkian D, Mur S, Cavatorta E, Pognon L, Rakza T, Storme L. Lower distending pressure improves respiratory mechanics in congenital diaphragmatic hernia complicated by persistent pulmonary hypertension. *J Pediatr.* (2018) 200:38–43. doi: 10.1016/j.jpeds.2018.04.027
28. te Pas AB, Siew M, Wallace MJ, Kitchen MJ, Fouras A, Lewis RA, et al. Establishing functional residual capacity at birth: the effect of sustained inflation and positive end-expiratory pressure in a preterm rabbit model. *Pediatr Res.* (2009) 65:537–41. doi: 10.1203/PDR.0b013e31819da21b
29. Wu Q, Liu J, Liu Y, Jiang Y. Management and experience of postural placement in postoperative mechanical ventilation of newborns. *Ann Palliat Med.* (2020) 9:1997–2002. doi: 10.21037/apm-20-1003
30. Hunt K, Dassios T, Ali K, Greenough A. Work of breathing at different levels of volume-targeted ventilation in infants with congenital diaphragmatic hernia. *Eur Respir J.* (2018) 52:PA4663. doi: 10.1183/13993003.congress-2018.PA4663
31. Saugstad OD, Sejersted Y, Solberg R, Wollen EJ, Bjoras M. Oxygenation of the newborn: a molecular approach. *Neonatology.* (2012) 101:315–25. doi: 10.1159/000337345
32. Lakshminrusimha S, Swartz DD, Gugino SF, Ma CX, Wynn KA, Ryan RM, et al. Oxygen concentration and pulmonary hemodynamics in newborn lambs with pulmonary hypertension. *Pediatr Res.* (2009) 66:539–44. doi: 10.1203/PDR.0b013e3181bab0c7
33. Victor VM, Rocha M, Esplugues JV, De la Fuente M. Role of free radicals in sepsis: antioxidant therapy. *Curr Pharm Des.* (2005) 11:3141–58. doi: 10.2174/1381612054864894
34. Thébaud B, Goss KN, Laughon M, Whitsett JA, Abman SH, Steinhorn RH, et al. Bronchopulmonary dysplasia. *Nat Rev Dis Primers.* (2019) 5:78. doi: 10.1038/s41572-019-0127-7
35. Wedgwood S, Steinhorn RH, Lakshminrusimha S. Optimal oxygenation and role of free radicals in PPHN. *Free Radic Biol Med.* (2019) 142:97–106. doi: 10.1016/j.freeradbiomed.2019.04.001
36. Paneth N. The evidence mounts against use of pure oxygen in newborn resuscitation. *J Pediatr.* (2005) 147:4–6. doi: 10.1016/j.jpeds.2005.04.058
37. Riley JS, Antiel RM, Rintoul NE, Ades AM, Waqar LN, Lin N, et al. Reduced oxygen concentration for the resuscitation of infants with congenital diaphragmatic hernia. *J Perinatol.* (2018) 38:834–43. doi: 10.1038/s41372-017-0031-5
38. Kashyap AJ, Crossley KJ, DeKoninck PL, Rodgers KA, Thio M, Skinner SM, et al. Neonatal cardiopulmonary transition in an ovine model of congenital diaphragmatic hernia. *Arch Dis Child Fetal Neonatal Ed.* (2019) 104:F617–23. doi: 10.1136/archdischild-2018-316045
39. Conforti A, Giliberti P, Landolfo F, Valfre L, Columbo C, Mondi V, et al. Effects of ventilation modalities on near-infrared spectroscopy in surgically corrected CDH infants. *J Pediatr Surg.* (2016) 51:349–53. doi: 10.1016/j.jpedsurg.2015.07.021
40. Putnam LR, Tsao K, Morini F, Lally PA, Miller CC, Lally KP, et al. Evaluation of variability in inhaled nitric oxide use and pulmonary hypertension in patients with congenital diaphragmatic hernia. *JAMA Pediatr.* (2016) 170:1188–94. doi: 10.1001/jamapediatrics.2016.2023
41. Group NINOS. Inhaled nitric oxide and hypoxic respiratory failure in infants with congenital diaphragmatic hernia. *Pediatrics.* (1997) 99:838–45. doi: 10.1542/peds.99.6.838
42. Cochius-den Otter S, Schaible T, Greenough A, van Heijst A, Patel N, Allegaert K, et al. The CoDiNOS trial protocol: an international randomised controlled trial of intravenous sildenafil versus inhaled nitric oxide for the treatment of pulmonary hypertension in neonates with congenital diaphragmatic hernia. *BMJ Open.* (2019) 9:e032122. doi: 10.1136/bmjopen-2019-032122
43. Lakshminrusimha S, Keszler M, Kirpalani H, Van Meurs K, Chess P, Ambalavanan N, et al. Milrinone in congenital diaphragmatic hernia - a randomized pilot trial: study protocol, review of literature and survey of current practices. *Matern Health Neonatol Perinatol.* (2017) 3:27. doi: 10.1186/s40748-017-0066-9
44. Davey M. Surfactant levels in congenital diaphragmatic hernia. *PLoS Med.* (2007) 4:e243. doi: 10.1371/journal.pmed.0040243
45. Van Meurs K, the Congenital Diaphragmatic Hernia Study Group. Is surfactant therapy beneficial in the treatment of the term newborn

infant with congenital diaphragmatic hernia? *J Pediatr*. (2004) 145:312–6. doi: 10.1016/j.jpeds.2004.04.056
46. Lally KP, Lally PA, Langham MR, Hirschl R, Moya FR, Tibboel D, et al. Surfactant does not improve survival rate in preterm infants with congenital diaphragmatic hernia. *J Pediatr Surg*. (2004) 39:829–33. doi: 10.1016/j.jpedsurg.2004.02.011
47. Colby CE, Lally KP, Hintz SR, Lally PA, Tibboel D, Moya FR, et al. Surfactant replacement therapy on ECMO does not improve outcome in neonates with congenital diaphragmatic hernia. *J Pediatr Surg*. (2004) 39:1632–7. doi: 10.1016/j.jpedsurg.2004.07.005
48. Boucherat O, Benachi A, Chailley-Heu B, Franco-Montoya ML, Elie C, Martinovic J, et al. Surfactant maturation is not delayed in human fetuses with diaphragmatic hernia. *PLoS Med*. (2007) 4:e237. doi: 10.1371/journal.pmed.0040237
49. Kurland Y, Gurung K, Pallotto EK, Manimtim W, Feldman K, Staggs VS, et al. Neurally adjusted ventilatory assist in neonates with congenital diaphragmatic hernia. *J Perinatol*. (2021) 41:1910–5. doi: 10.1038/s41372-021-01098-3
50. Amin R, Arca MJ. Feasibility of non-invasive neurally adjusted ventilator assist after congenital diaphragmatic hernia repair. *J Pediatr Surg*. (2019) 54:434–8. doi: 10.1016/j.jpedsurg.2018.05.011
51. Meinen RD, Alali YI, Al-Subu A, Wilhelm M, Wraight CL, McAdams RM, et al. Neurally-adjusted ventilatory assist can facilitate extubation in neonates with congenital diaphragmatic hernia. *Respir Care*. (2021) 66:41–9. doi: 10.4187/respcare.07681
52. Sturrock S, Ambulkar H, Williams EE, Sweeney S, Bednarczuk NF, Dassios T, et al. A randomised crossover trial of closed loop automated oxygen control in preterm, ventilated infants. *Acta Paediatr*. (2021) 110:833–7. doi: 10.1111/apa.15585
53. Hallenberger A, Poets CF, Horn W, Seyfang A, Urschitz MS. Closed-loop automatic oxygen control (CLAC) in preterm infants: a randomized controlled trial. *Pediatrics*. (2014) 133:e379–85. doi: 10.1542/peds.2013-1834
54. Gajdos M, Waitz M, Mendler MR, Braun W, Hummler H. Effects of a new device for automated closed loop control of inspired oxygen concentration on fluctuations of arterial and different regional organ tissue oxygen saturations in preterm infants. *Arch Dis Child Fetal Neonatal Ed*. (2019) 104:F360–5. doi: 10.1136/archdischild-2018-314769
55. Salverda H, Oldenburger N, Rijken M, Pauws S, Dargaville P, Te Pas A. The effect of automated oxygen control on clinical outcomes in preterm infants: a pre-and post-implementation cohort study. *Eur J Pediatr*. (2021) 180:2107–13. doi: 10.1007/s00431-021-03982-8
56. van den Heuvel M, van Zanten HA, Bachman TE, Te Pas AB, van Kaam AH, Onland W. Optimal target range of closed-loop inspired oxygen support in preterm infants: a randomized cross-over study. *J Pediatr*. (2018) 197:36–41. doi: 10.1016/j.jpeds.2018.01.077
57. Wise AC, Boutin MA, Knodel EM, Proudfoot JA, Lane BP, Evans ML, et al. Heliox adjunct therapy for neonates with congenital diaphragmatic hernia. *Respir Care*. (2018) 63:1147–53. doi: 10.4187/respcare.06079
58. Scarpelli EM, Condorelli S, Cosmi EV, Taylor FA. Lamb fetal pulmonary fluid. i. validation and significance of method for determination of volume and volume change. *Pediatr Res*. (1975) 9:190–5. doi: 10.1203/00006450-197504000-00010
59. Joe P, Wallen LD, Chapin CJ, Lee CH, Allen L, Han VK, et al. Effects of mechanical factors on growth and maturation of the lung in fetal sheep. *Am J Physiol Lung Cell Mol Physiol*. (1997) 272:L95–105. doi: 10.1152/ajplung.1997.272.1.L95
60. Garcia A, Stolar CJ. Congenital diaphragmatic hernia and protective ventilation strategies in pediatric surgery. *Surg Clin North Am*. (2012) 92:659–68. doi: 10.1016/j.suc.2012.03.003
61. Hirschl RB, Parent A, Tooley R, Shaffer T, Wolfson M, Bartlett RH. Lung management with perfluorocarbon liquid ventilation improves pulmonary function and gas exchange during extracorporeal membrane oxygenation (ECMO). *Artif Cells Blood Substit Immobil Biotechnol*. (1994) 22:1389–96. doi: 10.3109/10731199409138842
62. Hirschl RB, Philip WF, Glick L, Greenspan J, Smith K, Thompson A, et al. A prospective, randomized pilot trial of perfluorocarbon-induced lung growth in newborns with congenital diaphragmatic hernia. *J Pediatr Surg*. (2003) 38:283–9. doi: 10.1053/jpsu.2003.50095
63. American Academy of Pediatrics Section on Surgery, American Academy of Pediatrics Committee on Fetus and Newborn, Lally KP, Engle W. Postdischarge follow-up of infants with congenital diaphragmatic hernia. *Pediatrics*. (2008) 121:627–32. doi: 10.1542/peds.2007-3282
64. Koh JY, Jung E, Goo HW, Kim SC, Kim DY, Namgoong JM, et al. Functional and structural evaluation in the lungs of children with repaired congenital diaphragmatic hernia. *BMC Pediatr*. (2021) 21:120. doi: 10.1186/s12887-021-02586-3
65. Dao DT, Hayden LP, Buchmiller TL, Kharasch VS, Kamran A, Smithers CJ, et al. Longitudinal analysis of pulmonary function in survivors of congenital diaphragmatic hernia. *J Pediatr*. (2020) 216:158–64. doi: 10.1016/j.jpeds.2019.09.072

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REVIEW
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Unraveling the Genetics of Congenital Diaphragmatic Hernia: An Ongoing Challenge

Erwin Brosens[1], Nina C. J. Peters[2], Kim S. van Weelden[1,2,3], Charlotte Bendixen[4], Rutger W. W. Brouwer[5,6], Frank Sleutels[1], Hennie T. Bruggenwirth[1], Wilfred F. J. van Ijcken[5,6], Danielle C. M. Veenma[1,7], Suzan C. M. Cochius-Den Otter[3], Rene M. H. Wijnen[3], Alex J. Eggink[2], Marieke F. van Dooren[1], Heiko Martin Reutter[8,9], Robbert J. Rottier[3,6], J. Marco Schnater[3†], Dick Tibboel[3†] and Annelies de Klein[1†]*

[1] Department of Clinical Genetics, Erasmus MC Sophia Children's Hospital, Rotterdam, Netherlands, [2] Division of Obstetrics and Fetal Medicine, Department of Obstetrics and Gynecology, Erasmus MC Sophia Children's Hospital, Rotterdam, Netherlands, [3] Department of Pediatric Surgery and Intensive Care, Erasmus MC Sophia Children's Hospital, Rotterdam, Netherlands, [4] Unit of Pediatric Surgery, Department of General, Visceral, Vascular and Thoracic Surgery, University Hospital Bonn, Bonn, Germany, [5] Center for Biomics, Erasmus MC Sophia Children's Hospital, Rotterdam, Netherlands, [6] Department of Cell Biology, Erasmus MC Sophia Children's Hospital, Rotterdam, Netherlands, [7] Department of Pediatrics, Erasmus MC Sophia Children's Hospital, Rotterdam, Netherlands, [8] Institute of Human Genetics, University Hospital of Bonn, Bonn, Germany, [9] Neonatology and Pediatric Intensive Care, Department of Pediatrics and Adolescent Medicine, University Hospital Erlangen, Erlangen, Germany

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Tim Jancelewicz, University of Tennessee Health Science Center (UTHSC), United States

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***Correspondence:**
Erwin Brosens
e.brosens@erasmusmc.nl
orcid.org/0000-0001-8235-4010

† These authors have contributed equally to this work

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Congenital diaphragmatic hernia (CDH) is a congenital structural anomaly in which the diaphragm has not developed properly. It may occur either as an isolated anomaly or with additional anomalies. It is thought to be a multifactorial disease in which genetic factors could either substantially contribute to or directly result in the developmental defect. Patients with aneuploidies, pathogenic variants or *de novo* Copy Number Variations (CNVs) impacting specific genes and loci develop CDH typically in the form of a monogenetic syndrome. These patients often have other associated anatomical malformations. In patients without a known monogenetic syndrome, an increased genetic burden of *de novo* coding variants contributes to disease development. In early years, genetic evaluation was based on karyotyping and SNP-array. Today, genomes are commonly analyzed with next generation sequencing (NGS) based approaches. While more potential pathogenic variants are being detected, analysis of the data presents a bottleneck—largely due to the lack of full appreciation of the functional consequence and/or relevance of the detected variant. The exact heritability of CDH is still unknown. Damaging *de novo* alterations are associated with the more severe and complex phenotypes and worse clinical outcome. Phenotypic, genetic—and likely mechanistic—variability hampers ***individual*** patient diagnosis, short and long-term morbidity prediction and subsequent care strategies. Detailed phenotyping, clinical follow-up at regular intervals and detailed registries are needed to find associations between long-term morbidity, genetic alterations, and clinical parameters. Since CDH is a relatively rare disorder with only a few recurrent changes large cohorts of patients

are needed to identify genetic associations. Retrospective whole genome sequencing of historical patient cohorts using will yield valuable data from which today's patients and parents will profit Trio whole genome sequencing has an excellent potential for future re-analysis and data-sharing increasing the chance to provide a genetic diagnosis and predict clinical prognosis. In this review, we explore the pitfalls and challenges in the analysis and interpretation of genetic information, present what is currently known and what still needs further study, and propose strategies to reap the benefits of genetic screening.

Keywords: foregut, genetics, development, counseling, diaphragm, hernia, discordant monozygotic twin, congenital

INTRODUCTION

Congenital diaphragmatic hernia (CDH) [OMIM: 142340] has an estimated incidence of 1 in 1,750–5,880 live births (1–3) and is characterized by a defect of the diaphragm. This defect allows herniation of the abdominal organs into the thorax. CDH can be detected prenatally during first or second trimester ultrasounds in 50–68% of CDH pregnancies (4–7). Patients are often referred to a center of expertise with a specialized multidisciplinary team for prenatal assessment, prognostic and genetic counseling and care. CDH prevalence has slightly increased in the past years (3). Still, the mortality rates have decreased, probably due to better treatment strategies (8), although this decline is more pronounced in wealthier coutnries than in developing countries (9).

Most of what we know of human diaphragm development is based on descriptive and functional analyses of animal models. The diaphragm muscle develops initially from transient structures located at the top of the liver: the septum transversum, the pleuroperitoneal folds, the posthepatic mesenchymal plate, and the somites. Myoblast progenitors and other mesenchymal cells (10) in the developing pleuroperitoneal folds expand and migrate to the posthepatic mesenchymal plate. Vice versa, cells from the posthepatic mesenchymal plate migrate toward the pleuroperitoneal folds. Finally, the pleuroperitoneal folds fuse with the posthepatic mesenchymal plate between embryonic day (E) E12.5 and E13.5 (10, 11). When complete, this membrane separates the thoracic and abdominal cavity (E14.5). In CDH, this process is disrupted and the diaphragm will not fully close (12, 13). A more detailed description of diaphragm and CDH development can be found elsewhere in this issue (14).

Patients with aneuploidies, pathogenic single nucleotide variants, *de novo* Copy Number Variations (CNVs) (15–18) develop CDH, often in the form of a monogenetic syndrome and in combination with other anatomical malformations (2, 19). Here, we discuss what is currently known and inventoried what is necessary to provide optimal genetic counseling for the individual patients and their parents. We evaluate genetic outcome of a CDH cohort in the Erasmus MC-Sophia Children's Hospital, Rotterdam, the Netherlands, and propose strategies to reap the benefits of genetic screening.

CDH HAS SUBTYPES BASED ON DEFECT SIZE, TYPE AND ANATOMICAL LOCATION

CDH is the most severe diaphragm defects compared to other, less frequent defects such as incomplete muscularization of the diaphragm (diaphragmatic eventration) or the presence of just a thin layer of non-muscular tissue (sac hernia). Subtypes are identified by the size and anatomical location of the herniation. Most prevalent are Bochdalek hernias, which are mostly left-sided (20). Prenatal predictors for survival include associated malformations (21), defect size (7), lung volume (22), liver herniation (23), stomach position (24, 25), and lung-to-head ratio (26, 27). Other predictors include birth weight, Apgar score, respiratory parameters, cardiac anomalies, chromosomal changes, and pulmonary hypertension (28–30).

THE RELATION OF DEFECT SIZE AND GENETIC ALTERATIONS

Larger diaphragms defects are associated with a higher mortality rate, the prevalence of associated anatomical malformations as well as the number of associated anatomical malformations (21). We hypothesized that large continuous locus or gene changes (e.g., 15q26 loss, 17q12 loss; see **Table 1**) can modify multiple genes involved in diaphragm formation, and impact the development of the embryo in general. In contrast, small deletions or Single Nucleotide Variants (SNVs) as seen in for instance *FBN1*, *TGFB3*, and *SLC2A10* (see **Table 2**) will be associated with smaller defects. Therefore, we evaluated whether the size of the defect was associated with the finding of "a pathogenic genomic variant" and/or "a genetic syndrome." We compared the genetic test results and the defect size classification ($n = 336$). Statistical analysis did not indicate associations of the defect size with an different, uncommon genetic test result. What we did observed was that patients with no or little follow-up revealed associations ($P < 0.001$). In this category patients are present lacking a registered defect size or registered genetic test. This category includes patients who have not been subjected to an intervention due to intrauterine fetal demise or termination of pregnancy. In the Netherlands, pregnancies in which severe genetic anomalies (e.g., Edwards syndrome, Patau syndrome) or structural malformations are observed that are incompatible with

TABLE 1 | Pathogenic alterations in CDH patients of which the defect size was not registered.

Defect size (*n*)	Syndrome (*n*)	*n*	Death	Chromosome	Type	Inheritance	Zygosity	Genetic change
NR ($n = 41$)	Microdeletion	1	NR	3p26.3-p25.3	Loss	*de novo*	het	arr[hg18] 3p26.3-p25.3 (0–9398383) x1
	Microduplication		NR	11q23.3-q25	Gain	*de novo*	het	arr[hg18] 11q23.3-q25 (16192532–134452384) x3
	Microdeletion	1	NR	5p15	Loss	*de novo*	het	arr[hg19] 5p15 (0–37,299,510) x1,
	Microduplication		NR	12p13.3	Gain	*de novo*	het	arr[hg19] 12p13.31 (9,909,002–10,021,222) x 3
	Cornelia de lange	1	N	5p13.2	Missense	*de novo*	het	NM_1334333 (*NIPBL*): c.3574G>A; p. (Glu1192Lys)
	Microduplication	1	T	7q11.23	Gain	*de novo*	het	arr[hg18] 7q11.23 (72,701,018–74,143,000)
	Microduplication	1	NR	8p23	Gain	*ut*	het	46, XY, der (8) t (3;8) (p23; p23.1)
	Microduplication	1	D	9p24.3-p13.1	Gain	*de novo*	het	arr[hg18] 9p24.3p13.1 (0–39,155,853) x4, arr[hg18]9p13.1p11.2 (39,155,853–46,468,856) x3
	Microdeletion	1	T	9q31.1q31.2	Loss	*de novo*	het	arr[hg19] 9q31.1q31.2 (105,034,238–111,044,933) x1
	Trisomy 9	1	I	9	Aneuploidy	*de novo*	het	47, XX, +9(20)/46, XX (4)
	Mosaic MYRF gene	1	N	11q12.2	Splicing	*de novo*	het	NM_001127392.2 (*MYRF*): c.46+2T>C(r.spl?)
	Pallister Killian syndrome	3	T (1), NR (2)	12p10	Gain	*de novo*	het	47, XX/XY, +i (12) (p10)
	Microduplication	1	D	12q24.3	Gain	*ut*	het	46, XY, der (12) t (11,12) (q23.3; q24.3)
	Microdeletion	1	T	13q12	Loss	*de novo*	het	46, XY, del (13) (q12?) (8)/46, XY (35)
	Microdeletion	1	T	13q21.31q32.3	Loss	*de novo*	het	arr[hg19]13q21.31q32.3 (64,535,372–98,354,979) x1
	Patau syndrome	3	T (1), D (1), NR (1)	13	Aneuploidy	*de novo*	het	47, XX +13
	Isochromosome 14q	1	N	14q10	Gain	*de novo*	het	46, XX, i (14) (q10) (3)/46, XX (22)
	Microduplication	1	NR	15		*ut*	het	46, XX, der (15) t (2;15)
	Microdeletion	1	D	15q26	Loss	*de novo*	het	46, XY, t (1;14) (p22; q13), inv (6) (p25q22), del (15) (q26)
	Edward's syndrome	16	T (3), I (1), N (2), D (3), NR (9)	18	Aneuploidy	*de novo*	het	47 XX / XY + 18
	Down syndrome	1	NR	21	Aneuploidy	*de novo*	het	47, XX +21
	Cat eye syndrome	1	T	22q11.1q11.21	Gain	*de novo*	het	arr [hg19] 22q11.1q11.21 (14,449,498–17,017,139) x4
	XY reversal*	2	D (2)	XY	?	*de novo*	het	?

Genetic tests included karyotyping, SNP array or Whole exome sequencing. AR, Autosomal recessive; XLR, X-linked recessive; CH, compound heterozygote; n, number of patients; ut, unbalanced translocation.

life, are often terminated. The CDH defect size is not determined in those cases (see **Table 1**). Therefore, a complete genetic and phenotypic evaluation and subsequent association analysis in this particular group is difficult and often not performed.

ISOLATED CDH AND COMPLEX CDH

CDH may present as an isolated anomaly (isolated-CDH) or patients can have one or more additional anomalies (CDH-complex) (1, 31). Anomalies can be found in all body sites; cardiac anomalies, anomalies of the urogenital system, limb malformations, nervous system anomalies, orofacial clefts, and gastrointestinal anomalies including intestinal atresia (3, 32). Zaiss et al. described syndromic clinical features such as hypertelorism not assigned to a specific syndrome in 7.7% of studied patients (32). Pathogenic genetic alterations—both in complex and in isolated CDH—are associated with a worse prognosis (33). Moreover, *de novo* pathogenic alterations are seen more often in complex CDH (34–36). Phenotypical complex patients could be more likely to receive a genetic test. In our cohort, genetic test results were described for patients

TABLE 2 | Pathogenic alterations in CDH patients of which the defect size was registered.

Defect size (*n*)	Syndrome (*n*)	*n*	Death	Chromosome	Type	Inheritance	Zygosity	Genetic change
A ($n = 10$)	Wolf Hirschshorn Syndrome	1	NR	4p156.3	Loss	*de novo*	het	46, XY FISH: ish del (4) (p16.3p16.3) (D4S96-)
	Louys-Dietz syndrome V	1	NR	14q24	Frameshift	*AD*	het	NM_003239.4 (*TGFB3*): c.232del.G, p.(Glu78fs)
	Marfan syndrome	1	NR	15q21.1	Frameshift	*AD*	het	NM_000138.5 (*FBN1*):c1301_1302del, p.(Tyr434Serfs*17)
	Microdeletion	1	NR	16p13.3	Loss	*de novo*	het	46, XY arr[hg18] 16p13.3 (154,014–174,381) x1
	Arterial tortuosity syndrome	1	NR	20q13	Missense	*AR*	hom	NM_030777.4 (*SLC2A10*): c.127 6G>T, p.(Gly426Trp)
	Down syndrome	4	D (1), NR (3)	21	Aneuploidy	*de novo*	het	47, XX / 47, XY + 21
	Down syndrome	1	NR	21	Aneuploidy	*ut*	het	46, XY, t (15;21) (p12; p12)
B ($n = 4$)	Microduplication	1	NR	4p15.2p14	Gain	*de novo*	het	arr [hg18] 4p15.2p14 (224,500,018–38,700,366) x3
	Sotos syndrome	1	NR	5q35.2	Missense	*de novo*	het	NM_022455.5 (*NSD1*): c.5685C>G, p.(Cys1895Tyrp)
	Microduplication	1	NR	7q31.33–36.3	Gain	*de novo*	het	arr[hg19]7q31.33q36.3 (125839750_159124173) x3[0.2]/arr[hg19]7q31.33q36.3 (125839750_159124173) x4[0.1]
	Microdeletion	1	D	8p23.1	Loss	*de novo*	het	arr[hg18] 8p23.1 (8,139,051–12,619,015) x1
C ($n = 5$)	Fraser syndrome	1	NR	9p22.3	Splicing	*de novo*	het	NM_144966.7 (*FREM1*): c.5334 + 1G > A (r.spl?)
	Microdeletion			9p22.3	Loss	*Inherited*	het	arr[hg18] 9p22.3 (14,871,409–14,938,830) x1
	Prader Willi	1	NR	15q11	Gain	*de novo*	het	arr[hg18]15q11.2q13.1 (20,319,702–26,143,385) x3
	Microdeletion	1	NR	17q12	Loss	*de novo*	het	arr[hg19] 17q12 (34815551_36249430) x1
	Congenital disorder of glycosylation	1	NR	Xp11.23	Loss	*de novo*	het	NM_001042498 (*SLC35A2*): c.753delG, p.(Trp251fs)
	XY reversal	1	D	XY	?	*de novo*	?	–*
D ($n = 2$)	Microdeletion	1	N	15q26	Loss	*de novo*	het	arr[hg18] chr15:80,689,404–82,938,351 x 1 and
				17p12			het	arr[hg18] chr17:14049619–15497020 x1
	Microdeletion	1	D	22q11.2	Gain	*ut*	het	47, XY, +der (22) t (11;22) (q23.3; q11.2) mat

Genetic tests included karyotyping, SNP array or Whole exome sequencing. AR, Autosomal recessive; XLR, X-linked recessive; CH, compound heterozygote; n, number of patients; ut, unbalanced translocation.

with associated anomalies ($n = 207$) and for patients without associated anomalies ($n = 311$). Thus, there was not a priory bias in this respect ($p = 0.923$). Twenty patients with associated anomalies had pathogenic genetic alterations vs. one with isolated CDH ($P < 0.001$). Main outcome parameters of the Erasmus MC-Sophia Children's Hospital, Rotterdam, the Netherlands CDH cohort are depicted in **Tables 3**, **4**. Full cohort descriptions and analysis methods are described in **Supplementary Tables S1**, **S2**.

Comparing features of isolated CDH and complex CDH is difficult, depending on how accurately these two groups can be distinguished. Not all patients receive the same phenotypical evaluation and registration is sometimes incomplete. For instance, not all associated anatomical malformations are detectable with ultrasound. Nevertheless, increased resolution of prenatal ultrasound over time has improved the detection of associated anatomical malformations. Neurological symptoms could develop at later age and are not noticeable during the first months or years of development. Furthermore, not all symptoms observed during often organ specific evaluations of medical subspecialities. For instance, postnatal monitoring is essential to detect any associated neurological or ophthalmological symptoms. CDH registries would benefit from regular re-evaluation of these outcome measures. In short, there is a level of uncertainty in registries regarding which patients have no associated anomalies, have no associated anomalies detected, or have no associated anomalies registered.

TABLE 3 | Cohort description of output measures and genetic evaluation.

Group	Characteristic	Genetic test ($n = 530$)	No genetic test ($n = 275$)	Total (n)	P	Abnormal genetic test ($n = 62$)	No genetic test ($n = 275$)	No pathogenic changes ($n = 468$)	Total (n)	P
Sex	F	238^{a} (44.9%)	120^{a} (43.6%)	358 (44.5%)	0.824	34^{a} (54.8%)	120^{a} (43.6%)	204^{a} (43.6%)	358 (44.5%)	0.502
	M	285^{a} (53.8%)	150^{a} (54.5%)	435 (54.0%)		27^{a} (43.5%)	150^{a} (54.5%)	258^{a} (55.1%)	435 (54.0%)	
	O	7^{a} (1.3%)	5^{a} (1.8%)	12 (1.5%)		1^{a} (1.6%)	5^{a} (1.8%)	6^{a} (1.3%)	12 (1.5%)	
Associated anomalies	CDH-C	207^{a} (39.1%)	104^{a} (37.8%)	311 (38.6%)	0.923	56^{a} (90.3%)	104^{b} (37.8%)	151^{b} (32.3%)	311 (38.6%)	4.5658E-16
	CDH-I	311^{a} (58.7%)	164^{a} (59.6%)	475 (59.0%)		6^{a} (9.7%)	164^{b} (59.6%)	305^{b} (65.2%)	475 (59.0%)	
	CDH-MD	12^{a} (2.3%)	7^{a} (2.5%)	19 (2.4%)		0^{a} (0.0%)	7^{a} (2.5%)	12^{a} (2.6%)	19 (2.4%)	
Location of defect	Bilateral	4^{a} (0.8%)	6^{a} (2.2%)	10 (1.2%)	0.005998	0^{a} (0.0%)	6^{a} (2.2%)	4^{a} (0.9%)	10 (1.2%)	0.004092
	Eventration	17^{a} (3.2%)	1^{b} (0.4%)	18 (2.2%)		$1^{a,b}$ (1.6%)	1^{b} (0.4%)	16^{a} (3.4%)	18 (2.2%)	
	Left	415^{a} (78.3%)	199^{a} (72.4%)	614 (76.3%)		48^{a} (77.4%)	199^{a} (72.4%)	367^{a} (78.4%)	614 (76.3%)	
	POE	4^{a} (0.8%)	2^{a} (0.7%)	6 (0.7%)		2^{a} (3.2%)	2^{a} (0.7%)	2^{a} (0.4%)	6 (0.7%)	
	Right	73^{a} (13.8%)	58^{b} (21.1%)	131 (16.3%)		$7^{a,b}$ (11.3%)	58^{b} (21.1%)	66^{a} (14.1%)	131 (16.3%)	
	MD	17^{a} (3.2%)	9^{a} (3.3%)	26 (3.2%)		4^{a} (6.5%)	9^{a} (3.3%)	13^{a} (2.8%)	26 (3.2%)	
Defect size	A	97^{a} (18.3%)	19^{b} (6.9%)	116 (14.4%)	1.3023E-41	$10^{a,b}$ (16.1%)	19^{b} (6.9%)	87^{a} (18.6%)	116 (14.4%)	1.3224E-44
	B	50^{a} (9.4%)	2^{b} (0.7%)	52 (6.5%)		4^{a} (6.5%)	2^{b} (0.7%)	46^{a} (9.8%)	52 (6.5%)	
	C	157^{a} (29.6%)	12^{b} (4.4%)	169 (21.0%)		5^{a} (8.1%)	12^{a} (4.4%)	152^{b} (32.5%)	169 (21.0%)	
	D	32^{a} (6.0%)	0^{b} (0.0%)	32 (4.0%)		2^{a} (3.2%)	0^{b} (0.0%)	30^{a} (6.4%)	32 (4.0%)	
	NR	194^{a} (36.6%)	242^{b} (88.0%)	436 (54.2%)		41^{a} (66.1%)	242^{b} (88.0%)	153^{c} (32.7%)	436 (54.2%)	
Timing of test	MD-genetic test	–	–	–	–	13^{a} (21.0%)	0^{b} (21.0%)	88^{a} (18.8.0%)	101 (12.5%)	8.4554E-167
	MD-no genetic test	–	–	–		0^{a} (0.0%)	127^{b} (46.2%)	0^{a} (0.0%)	127 (15.8%)	
	Postnatal-genetic test	–	–	–		16^{a} (25.8%)	0^{b} (0%)	101^{a} (21.6%)	117 (14.5%)	
	Postnatal-no genetic test	–	–	–		0^{a} (0.0%)	96^{b} (34.9%)	0^{a} (0.0%)	96 (11.9%)	
	Prenatal-genetic test	–	–	–		33^{a} (53.2%)	0^{b} (0%)	279^{a} (59.6%)	312 (38.8%)	
	Prenatal-no genetic test	–	–	–		0^{a} (0.0%)	52^{b} (18.9%)	0^{a} (0.0%)	52 (6.5%)	

In total, 530 out of 805 patients received a genetic test. Defect size (A–D) was described in 369 patients. Defect sizes are classified from A to D as described in the method section. A is the smallest defect size and D a (near) absence of the diaphragm. Within a column each characteristic that does not share a subscript letter ($^{a-b}$) differs significantly from those with different subscript letters ($^{a-b}$) whose column proportions do not differ significantly from each other at the 0.05 level. For instance, more patients with associated anomalies have an abnormal test and vice versa more patients with an isolated defect have no abnormal test ($P < 0.001$). Patients with defect size A stand apart from the other defect sizes in respect to the number of abnormal genetic tests, C in having no genetic test and having no pathogenic alteration ($P < 0.001$). There are differences in having no genetic test, having an abnormal test result and having a normal test result comparing post- and pre-natal subgroups ($P < 0.001$). Trisomy 13, 18, and 21 were evaluated in 530 patients and more than half of the patients received at least karyotyping or SNP-array. A full cohort description is available in ***Supplementary Table S1****. Complete statistical comparison of patients with a genetic test is depicted in* ***Supplementary Table S2****. MD, Missing data; CDH-C, CDH patients with associated defects; CDH-I, CDH patients without other associated defects; CDH-MD, CDH patients in which no additional information was registered; POE, Paraoesophageal hernia; EV, Eventration; BL, Bilateral hernia; AGT, abnormal genetic test; NPC, no pathogenic changes.*

GENETIC ASSOCIATIONS AND CO-MORBIDITY

Long-term complications in children born with CDH include chronic lung disease, feeding difficulties, gastro-esophageal reflux, growth failure, scoliosis, chest asymmetry, neurodevelopmental delay, and sensorineural hearing loss (37, 38). These co-morbidities can be either a direct or indirect consequence of the CDH or be a consequence of the treatment. Damaging *de novo* variations in both isolated CDH and complex CDH-complex have been found associated with pulmonary hypertension, higher mortality rate, and worse neurodevelopmental outcome (33). There is a large difference in survival rates between patients with or without persistent pulmonary hypertension (39) and bronchopulmonary sequestration (40). The genetic contribution to bronchopulmonary sequestration etiology is unknown. Mutations in *BMPR2* (41, 42) and several *SMAD* signaling molecule genes have been associated with the development of pulmonary hypertension in adults and children (43–45). A striking association between TGF-β/SMAD signaling and pulmonary hypertension has been reported in CDH, as the CDH lungs had increased miR-200b expression and decreased TGF-β/SMAD signaling (46). Increasing miR-200b decreases the TGF-β signaling and reduces lung hypoplasia in a nitrofen induced congenital diaphragmatic hernia -pulmonary hypertension rat model (46). Similarly, Pereira-Terra and colleagues described a specific micro-RNA signature in tracheal aspirate fluid, upregulation of miR-200b and miR-10a and decreased TGFB signaling (47). Patients with mutations in genes

TABLE 4 | Significant differences in output measures of patients with a genetic test.

Group	Characteristic	Abnormal genetic test ($n = 62$)	No pathogenic changes ($n = 468$)	*P*
Associated anomalies	CDH-complex ($n = 207$)	56[a] (27.1%)	151[a] (72.9%)	1,432E-14
	CDH-isolated ($n = 311$)	6[b] (1.9%)	305[b] (98.1%)	
	CDH-unknown ($n = 12$)	0[a,b] (0.0%)	12[a,b] (100.0%)	
Defect size	A ($n = 97$)	10[a,b] (10.3%)	87[a,b] (89.7%)	0.000006
	B ($n = 50$)	4[a,b] (8.0%)	46[a,b] (92.0%)	
	C ($n = 157$)	5b (3.2%)	152[b] (96.8%)	
	D ($n = 32$)	2[a,b] (6.3%)	30[a,b] (93.8%)	
	NR ($n = 194$)	41[a] (21.1%)	153[a] (78.9%)	
Type of genetic test	Karyotyping	297 (56.0%)		
	WES	51 (9.6%)		
	Array	362 (68.3%)		
	Trisomy 13, 18, 21*	530 (100%)		

Significant differences when evaluating only patients with a genetic test. Trisomy 13, 18, and 21 were evaluated in 530 patients and more than half of the patients received at least karyotyping or SNP-array. An abnormal genetic test is seen more often in complex-CDH ($P < 0.001$) and defect size C differs from the missing data category ($P < 0.001$) as substantially more abnormal genetic tests are described in the later. Within a column each characteristic measure that does not share a subscript letter ($^{a-b}$) differs significantly from those with different subscript letters ($^{a-b}$) whose column proportions do not differ significantly from each other at the 0.05 level. WES, whole exome sequencing; MD, Missing data; CDH-C, CDH patients with associated defects; CDH-I, CDH patients without other associated defects; CDH-MD, CDH patients in which no additional information was registered; POE, Paraoesophageal hernia; EV, Eventration; BL, Bilateral hernia; AGT, abnormal genetic test; NPC, no pathogenic changes.

from this pathway have connective tissue disorders (48). In patients and mice, several genetic factors have been associated to lung and cardiac abnormalities (2, 49–52). CDH has been found in patients with connective tissue disorders such as Marfan syndrome (53), Loeys-Dietz Syndrome (54, 55) and arterial tortuosity syndrome (56). Patients with these connective tissue disorders are at increased risk of cardiovascular problems (57, 58) later in life. Abnormal retinoic acid signaling can result in a diaphragm defect (59). Patients with variants in *STRA6* and *RARB* -receptors and deletions of *RBP1* at chromosome 3q22 (60, 61) in the retinoic acid signaling pathway have ophthalmic symptoms (62, 63). Patients with CDH may have other eye defects as well (64, 65). These occurrences of direct genotype-phenotype correlations stress the importance of genetic diagnostic screening to inform parents and patients about possible co-morbidities.

CDH IS A COMPLEX GENETIC DISORDER

CDH is a multifactorial disease but neither environmental nor genetic contributions have been fully characterized. Maternal morbidities during pregnancy such as pre-gestational hypertension (66) and pre-existent maternal obesity (67–69) are associated with an increased risk for development of CDH in the fetus. Several other environmental factors have been associated with an increased risk: antidepressant medication (70), antibacterial medication (71), exposure to fungicides (72), the immunosuppressant drug mycophenolate mofetil (73), methotrexate use (74), exposure to cadmium (75), pesticides (76), hairspray use (77), alcohol intake (69, 77–79), and smoking (75, 78, 80). However, to what extent these associations impact diaphragm development and the onset of CDH is not known. The mother's nutrient intake during pregnancy is associated as well (81, 82); reduced vitamin A intake during pregnancy has the strongest associations with CDH (83, 84). Vitamin A shortage can be detected postnatally (85). It is hard to determine whether environmental factors explain some of the non-genetic contributions on a population level or to what extent the environment interacts with the processes disturbed by genetic anomalies. Epigenetic differences acquired during the life span can be detected between monozygotic twin pairs (86–88). Evaluating these differences—and the resulting gene expression changes—is an interesting approach. There are methods to overcome cellular heterogeneity and if epigenetic changes are present in blood these can be compared between patient and sibling (89–91).

The exact heritability—the contribution of genetic factors—is difficult to determine, in light of the relatively low disease incidence, the high mortality limiting vertical transmission and the limited numbers of twin pregnancies (92, 93). Heritability can be estimated using twin studies. For CDH, the concordance rates in dizygotic and monozygotic twins are comparable. Fifty-three monozygotic twins have been described, of whom 12 were concordant for CDH (2, 92). In our cohort, 24 twin pairs (15 dizygotic, 8 monozygotic, and one same sex twin pair of whom no genetic material was available to determine zygosity) are described. One dizygotic and one monozygotic twin pair were concordant for CDH. To reduce the effect of technical noise in twin comparisons, we used different alignment techniques, variant callers and statistics (see **Supplementary Table S3**). Neither the larger CNVs (94) nor SNVs (see **Supplementary Table S3**) differed between these twin siblings. Differences in phenotype can also be the result of twin-to-twin perfusion differences. Furthermore, single nucleotide changes could be located outside the coding sequence or at very low frequency, and then could not be detected with exome sequencing.

Somatic mosaicism is difficult to determine when the affected tissue or cells are missing. The mutated diaphragmatic cells might not have survived in sufficient quantities and, therefore, be undetectable with sequencing technologies (95). In line with this, whole genome sequencing did not find causative somatic variants in diaphragm biopsies (96, 97). In contrast, germline *de novo* variants are often present (33, 96–98). Females have a higher burden of *de novo* variants (98), suggesting a female protective model. Large cohort descriptions about sibling recurrence rate (92) or familial CDH are not available. In our cohort, only a few familial cases are known (<1%). Still, CDH is described to segregate through families (1) and/or present as a monogenetic disorder following autosomal dominant (53, 98–109), autosomal recessive (62, 110), or X-linked (111–113) inheritance patterns.

Depending on the specific family the monogenetic disorder has CDH is either a common or a less prevalent feature. More than 100 (candidate) genes have been described, mostly identified from animal models or monogenetic syndromes (2, 19). Monogenetic syndromes often have distinct phenotypical features and have been reviewed by Longoni et al. and Yu et al. (20, 114). Monogenetic syndromes in which CDH is a frequent feature are, for instance, autosomal recessive Donnai Barrow syndrome (OMIM: #22248, *LRP2* gene), syndromic microphthalmia (#601186, #615524, *STRA6*, *RARB*), and autosomal dominant cardiac-urogenital syndrome (#3618280, *MYRF* gene). Associated phenotypes in these syndromes are congenital heart defects, sensorineural hearing loss, microphthalmia, genitourinary malformations, craniosynostosis and myopia with each of these syndromes its distinct features. Detailed phenotyping might be crucial in diagnosing clusters of CDH patients: either "phenotype first" and searching for an overlapping gene or "genotype first" and searching if patients with the same affected gene have an overlapping phenotype. Interestingly, Fryns syndrome and also Pentalogy of Cantrell have CDH as a defining feature; yet the gene or genes responsible for these conditions are not yet known.

CNV studies reported overlapping deletions and duplications, such as duplications of 11q23-qter (115), 16p11.2p duplications (15, 18), 17q12 deletions (15, 18, 116, 117), and 5p15.2 deletions (15). By prioritizing and sequencing the genes within these CNVs in other patients, new disease genes have been discovered. For example, in the 8p23.1 deletions (118–120), *GATA4* (50) and *SOX7* (121) and in case of 8q23.1 deletions (18), *ZFPM2* (122) are the genes likely contributing to CDH. 15q26 deletions (120, 123) and subsequent sequencing implicate *NR2F2* as a disease gene (124). For 1q41–1q42 deletions, one duplication disrupting the *HLX* gene and subsequent *HLX* gene variants have been described (15, 18, 125–128). Constraint coding regions are enriched for *de novo* variants (104), and using variant evaluation guidelines of rare *de novo* changes in these types of constraint genes (129) result in a likely pathogenic or pathogenic classification, especially if variants result in reduced amounts of protein.

Interpretation of genetic results can be hindered by reduced penetrance (18, 122) and variable expressivity (2) that may mask the causal culprit in segregation analysis (see **Figure 1**). Polygenic inheritance (51), locus heterogeneity (33, 34, 130), and contributions of different kinds of genetic variation (17, 114) mask culprits from innocent bystanders. Therefore, large patient and control samples sizes are required to have enough power to classify variants into "benign," "causal," or "contributing."

FROM PATHOGENIC ALTERATION TO CDH

Finding a genetic variant predicted to be deleterious is only the first step in proving the functional effect of this DNA alteration. This is especially true for missense changes, in-frame insertion-deletions and copy number variations. Often there is only *in-silico* evidence regarding the impact of a variant on gene function and the way in which the disturbed gene function affects a biological pathway or mechanism. What is lacking is proof how a specific deleterious variant lead to defective diaphragm formation. Unfortunately, for most likely pathogenic CNVs and SNVs, the assumed functional consequence is based on the genetic alteration itself: i.e., copy number loss or nonsense variant is assumed to result in reduced amounts of mRNA expression and protein. Deleterious *de novo* missense variants and in-frame insertion-deletions in conserved coding regions are more difficult to relate to a likely functional consequence and is often on *in-silico* surveys. Improving the *in-vitro* evaluation of candidate variants is crucial in distinguishing causal variants from non-causal variants. These experiments require tremendous effort and can be complicated by the presence of more than one candidate alteration.

Detecting a deleterious variant in a gene in multiple patients helps prioritizing candidate genes for function evaluation and studies using animal models. In a large cohort ($n = 827$), seven syndromic and four recurrent CNVs were identified (104). Some of these have already been associated with CDH; e.g., 17q12 deletions, 16p13.1 duplications, 22q11 deletions, and 21q22 duplications. Furthermore, 87 CNVs were *de novo*, of which 54 were large (>2 Mb) deletions (104). Although non-recurrent, at least a proportion of these large *de novo* deletions are likely to be related to the patient's phenotype. Ten genes were enriched for *de novo* variants, of which mitochondrial lon peptidase 1 (*LONP1*) and Aly/REF export factor (*ALYREF*) were the most promising candidate disease genes. *LONP1*, *MYRF* as well as *ZFPM2* reached or approached genome wide significance when a variant burden test was performed for all deleterious changes (i.e., including inherited variants) (104). Combining multiple "omics" and *in-vitro* translational approaches can potentially bridge the gap between genetic findings and animal models.

In animal models, fewer progenitors reaching the PPF at the proper developmental due to decreased proliferation, increased apoptosis, migration defects or failure to differentiate in their proper cell fates have been proposed as causes for CDH (131–134). Disturbances in specific processes such as retinoic acid signaling or muscle connective tissue formation were initially discovered in animal experiments; genes associated with these pathways or processes were subsequently found altered in patients (132, 135–138). Additionally, disturbed processes can be identified using gene enrichment strategies to find common denominators in the affected genes and loci. Longoni and colleagues described the enrichment of rare, likely deleterious variants in CDH patients of genes derived from mouse PPF embryonic transcriptomes (139), known human disease genes, their protein interaction partners and candidate genes from CNV hotspots (35). Often, these alterations were inherited and implicate non-Mendelian inheritance patterns. On the individual level, these changes can be regarded as risk factors. Combined, these changes may affect a biological pathway to such an extent that they result in CDH. Assigning such a pathway or process—for instance how these gene variants disturb myoblast progenitor cell proliferation or migration—is not easy. Animal models are not perfect, although they provide evidence of involvement of a gene when it is knocked-out and in which cases the animals develop CDH at a certain frequency. However, this

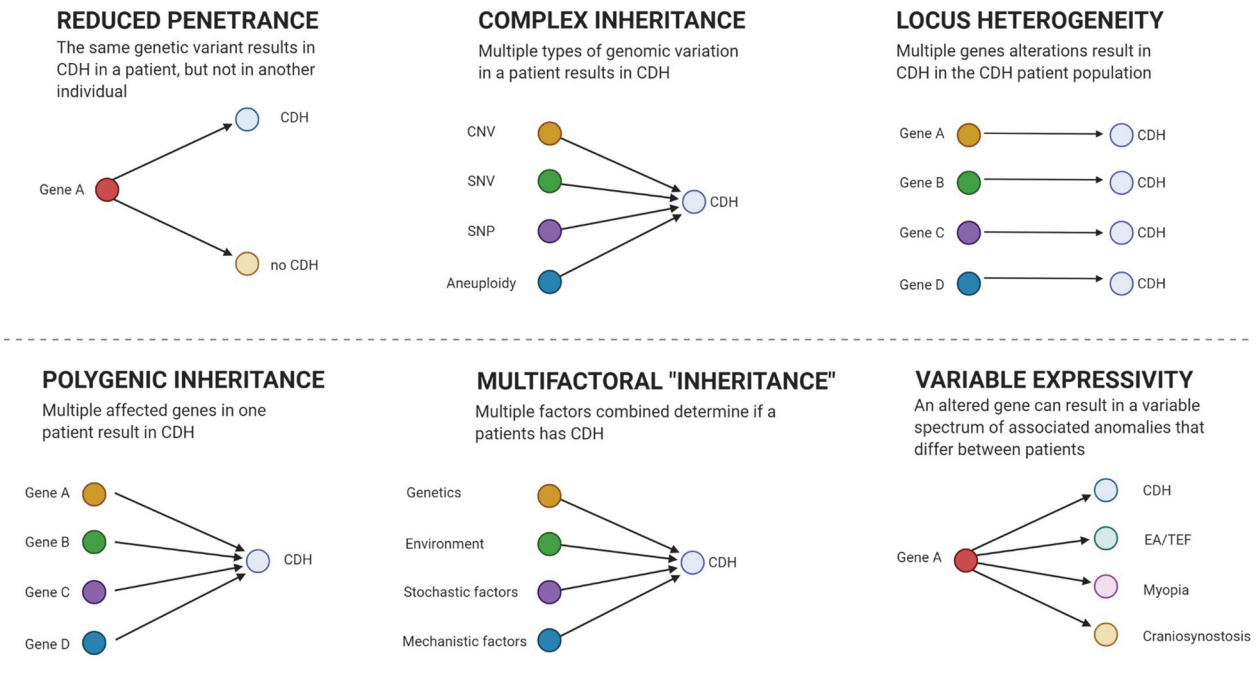

FIGURE 1 | Genetic models. Figure created with BioRender.com.

procedure hardly ever takes into account that genetic variation is mostly not a complete loss-of-function of a gene. Missense variants, copy number gains and heterozygous changes could—and likely do—differ in impact or mechanism of action. Thus, in these cases, knock-out models either over- or underestimate the effect of a genetic variant.

In some cases, specific variants can be associated with the causative mechanism; e.g., the association of *FBN1* variants in Marfan syndrome (53) and defects in the connective tissue. Indeed, our cohort included patients with *FBN1* and *TGFB3* alterations. In other patients, the affected pathway is known; e.g., patients with deletions of *NR2F2* (123) have a defect in a gene that codes for a receptor that is activated by retinoic acid signaling (140). Of other genes, we know that they interact with other disease genes, are expressed in the developing diaphragm and are also associated with retinoic acid signaling (e.g., *ZFPM2*, *GATA4*). A small difference in spatial and temporal binding and organ-specific combination of transcription factors have been suggested as links between the different syndromes with CDH (141). Most of the deleterious CNVs and aneuploidies are assumed pathogenic and the most likely cause of the diaphragm defect. However, how these—often continuous gene deletions—in patients impact diaphragm formation and subsequently result in CDH remains unclear.

TEMPORAL SCREENING BIAS

Technologies have a different resolution to detect genomic changes ranging from chromosome arms, several mega-bases to single nucleotide level. Initially, patients were evaluated with karyotyping, MLPA and QF-PCR, with which only aneuploidies or chromosome (band) level changes could be detected. At the Erasmus MC-Sophia Children's Hospital, SNP-array was introduced in 2010 and is standard practice in case of ultrasound abnormalities since 2012. The use of SNP arrays increased the detection resolution to gains and losses of several from mb to kilobases. Many patients in our cohort have retrospectively been re-evaluated with SNP-array. In 10.9% of patients a pathogenic change was. Similarly, 10.4% of patients registered in the EUROCAT registry (1980–2009) have a chromosomal anomaly, genetic syndrome or microdeletion (3). This was before the NGS era, and the findings mostly represent the larger genetic changes with a large phenotypic effect. Whole exome sequencing was introduced in our clinic more recently (2015), and initially only used to evaluate the more complex patients. Restoring the temporal screening bias by screening large historical cohorts of patients and subsequent evaluating potential associations between genetic factors and long-term morbidity can benefit the future and today's patients and parents.

COLLABORATION IS KEY

Combining disease cohorts revealed that damaging *de novo* alterations are associated with the more severe and complex phenotypes (33, 130). This strategy was pivotal in identifying disease genes (98, 104, 130, 142). The success of this effort stresses the importance of collaborations such as the DHREAMS

consortium (http://www.cdhgenetics.com). Trio whole genome-based approaches are recommended, as these enable to simultaneously determine different types of genetic variation. Additionally, this technique is suited for continuous re-analysis. By combining and sequencing these cohorts, the CDH-EURO consortium (143) and Congenital Diaphragmatic Hernia Study Group (144) can add to endeavors of the DHREAMS consortium. This will enable to identify genes that are more often affected in patients than by chance alone, and will allow manageable numbers of required functional tests and animal models. For collaborations to work, samples need to be stored in well-managed biobanks and data should be meticulously archived for later re-analysis or re-evaluation. New challenges for these biobanks and data archiving and sharing are privacy regulations (145). Sharing of patient material and data should consider the privacy of participants and their families but also acknowledge the efforts of stakeholders such as researchers and clinicians (146). An ethical and legal balance should be sought weighing the privacy needs of individual patients against the medical benefits of the patient population.

CONCLUSIONS

Diagnostic yields of up to 37% using next generation sequencing have been proposed. These yields are reached when, in addition to genes from known monogenetic syndromes, heterozygous *de novo* variants in genes expressed at the proper time-point in relevant tissue in animal models are classified as likely pathogenic (105). Importantly, heritability and diagnostic yield are calculated on a population level. From a patient's or parents' perspective it matters the most to know (1) if they themselves or ***their*** children **have** *or* **do not have** genetic changes in their genome explaining the CDH, (2) if subsequent children or patients' offspring are at risk of CDH, and (3) what the consequences of these changes are for the prognosis and/or the probability of complications. CDH is now mostly detected prenatally; consequently, fast, accurate, and predictive genetic diagnostics are increasingly needed. As about a third of patients have a *de novo* variant in the coding region (104). For parents to make informed choices, it is vital to knowing if a genetic variant detected in their child is causal or benign, and what the predicted consequences are of this variant.

AUTHOR CONTRIBUTIONS

EB, AdK, and DT: conceptualization and funding acquisition. RB and WvI: methodology and software. EB: validation, visualization, and project administration. KvW, SO, EB, and RB: formal analysis. EB and RB: investigation. AE, DT, and RW: resources. EB, RB, NP, and DV: data curation. EB and KvW: writing—original draft preparation. NP, AdK, MvD, SO, RW, AE, DT, RR, CB, HR, DV, WvI, FS, HB, RB, and JS: writing—review and editing. AdK and EB: supervision. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.800915/full#supplementary-material

REFERENCES

1. Pober BR. Overview of epidemiology, genetics, birth defects, chromosome abnormalities associated with CDH. *Am J Med Genet C Semin Med Genet.* (2007) 145c:158–71. doi: 10.1002/ajmg.c.30126
2. Kardon G, Ackerman KG, McCulley DJ, Shen Y, Wynn J, Shang L, et al. Congenital diaphragmatic hernias: from genes to mechanisms to therapies. *Dis Models Mech.* (2017) 10:955–70. doi: 10.1242/dmm.028365
3. McGivern MR, Best KE, Rankin J, Wellesley D, Greenlees R, Addor MC, et al. Epidemiology of congenital diaphragmatic hernia in Europe: a register-based study. *Arch Dis Child Fetal Neonatal Ed.* (2015) 100:F137–44. doi: 10.1136/archdischild-2014-306174
4. Grandjean H, Larroque D, Levi S. The performance of routine ultrasonographic screening of pregnancies in the Eurofetus Study. *Am J Obstetr Gynecol.* (1999) 181:446–54. doi: 10.1016/S0002-9378(99)70577-6
5. Graham G, Devine PC. Antenatal diagnosis of congenital diaphragmatic hernia. *Semin Perinatol.* (2005) 29:69–76. doi: 10.1053/j.semperi.2005.04.002
6. Garne E, Haeusler M, Barisic I, Gjergja R, Stoll C, Clementi M. Congenital diaphragmatic hernia: evaluation of prenatal diagnosis in 20 European regions. *Ultrasound Obstetr Gynecol.* (2002) 19:329–33. doi: 10.1046/j.1469-0705.2002.00635.x
7. Burgos CM, Frenckner B, Luco M, Harting MT, Lally PA, Lally KP. Prenatally versus postnatally diagnosed congenital diaphragmatic hernia - side, stage, and outcome. *J Pediatric Surg.* (2019) 54:651–5. doi: 10.1016/j.jpedsurg.2018.04.008
8. Gupta VS, Harting MT, Lally PA, Miller CC, Hirschl RB, Davis CF, et al. Congenital diaphragmatic hernia study, mortality in congenital diaphragmatic hernia: a multicenter registry study of over 5000 patients over 25 years. *Ann Surg.* (2021). doi: 10.1097/SLA.0000000000005113. [Epub ahead of print].
9. Global PaedSurg Research C. Mortality from gastrointestinal congenital anomalies at 264 hospitals in 74 low-income, middle-income, and high-income countries: a multicentre, international, prospective cohort study. *Lancet.* (2021) 398:325–39. doi: 10.1016/S0140-6736(21)00767-4
10. Cleal L, McHaffie SL, Lee M, Hastie N, Martínez-Estrada OM, Chau YY. Resolving the heterogeneity of diaphragmatic mesenchyme: a novel mouse model of congenital diaphragmatic hernia. *Dis Models Mech.* (2021) 14:dmm046797. doi: 10.1242/dmm.046797

11. Carmona R, Cañete A, Cano E, Ariza L, Rojas A, Muñoz-Chápuli R. Conditional deletion of WT1 in the septum transversum mesenchyme causes congenital diaphragmatic hernia in mice. *Elife.* (2016) 5:e16009. doi: 10.7554/eLife.16009
12. Sefton EM, Gallardo M, Kardon G. Developmental origin and morphogenesis of the diaphragm, an essential mammalian muscle. *Dev Biol.* (2018) 440:64–73. doi: 10.1016/j.ydbio.2018.04.010
13. Sefton EM, Kardon G. Connecting muscle development, birth defects, and evolution: an essential role for muscle connective tissue. *Curr Top Dev Biol.* (2019) 132:137–76. doi: 10.1016/bs.ctdb.2018.12.004
14. Edel GG, Schaaf G, Wijnen RMH, Tibboel D, Kardon G, Rottier RJ. Cellular origin(s) of congenital diaphragmatic hernia. *Front Pediatrics.* (2021) 9:804496. doi: 10.3389/fped.2021.804496
15. Zhu Q, High FA, Zhang C, Cerveira E, Russell MK, Longoni M, et al. Systematic analysis of copy number variation associated with congenital diaphragmatic hernia. *Proc Natl Acad Sci USA.* (2018) 115:5247–52. doi: 10.1073/pnas.1714885115
16. Scott DA, Klaassens M, Holder AM, Lally KP, Fernandes CJ, Galjaard RJ, et al. Genome-wide oligonucleotide-based array comparative genome hybridization analysis of non-isolated congenital diaphragmatic hernia. *Human Mol Genet.* (2007) 16:424–30. doi: 10.1093/hmg/ddl475
17. Yu L, Wynn J, Ma L, Guha S, Mychaliska GB, Crombleholme TM, et al. *De novo* copy number variants are associated with congenital diaphragmatic hernia. *J Med Genet.* (2012) 49:650–9. doi: 10.1136/jmedgenet-2012-101135
18. Wat MJ, Veenma D, Hogue J, Holder AM, Yu Z, Wat JJ, et al. Genomic alterations that contribute to the development of isolated and non-isolated congenital diaphragmatic hernia. *J Med Genet.* (2011) 48:299–307. doi: 10.1136/jmg.2011.089680
19. Holder AM, Klaassens M, Tibboel D, de Klein A, Lee B, Scott DA. Genetic factors in congenital diaphragmatic hernia. *Am J Hum Genet.* (2007) 80:825–45. doi: 10.1086/513442
20. Longoni M, Pober BR, High FA. Congenital diaphragmatic hernia overview. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, editors. *GeneReviews(®).*Seattle, WA: University of Washington; Copyright © 1993-2020. Seattle, WA: University of Washington. *GeneReviews Is a Registered Trademark of the University of Washington, Seattle* (1993).
21. Congenital Diaphragmatic Hernia Study G, Morini F, Valfrè L, Capolupo I, Lally KP, Lally PA, et al. Congenital diaphragmatic hernia: defect size correlates with developmental defect. *J Pediatric Surg.* (2013) 48:1177–82. doi: 10.1016/j.jpedsurg.2013.03.011
22. Kilian AK, Büsing KA, Schuetz EM, Schaible T, Neff KW. Fetal MR lung volumetry in congenital diaphragmatic hernia (CDH): prediction of clinical outcome and the need for extracorporeal membrane oxygenation (ECMO). *Klinische Padiatrie.* (2009) 221:295–301. doi: 10.1055/s-0029-1192022
23. Metkus AP, Filly RA, Stringer MD, Harrison MR, Adzick NS. Sonographic predictors of survival in fetal diaphragmatic hernia. *J Pediatric Surg.* (1996) 31:148–51; discussion: 151–2. doi: 10.1016/S0022-3468(96)90338-3
24. Cordier AG, Jani JC, Cannie MM, Rodó C, Fabietti I, Persico N, et al. Stomach position in prediction of survival in left-sided congenital diaphragmatic hernia with or without fetoscopic endoluminal tracheal occlusion. *Ultrasound Obstetr Gynecol.* (2015) 46:155–61. doi: 10.1002/uog.14759
25. Basta AM, Lusk LA, Keller RL, Filly RA. Fetal stomach position predicts neonatal outcomes in isolated left-sided congenital diaphragmatic hernia. *Fetal Diagnosis Ther.* (2016) 39:248–55. doi: 10.1159/000440649
26. Deprest JA, Flemmer AW, Gratacos E, Nicolaides K. Antenatal prediction of lung volume and *in-utero* treatment by fetal endoscopic tracheal occlusion in severe isolated congenital diaphragmatic hernia. *Semin Fetal Neonatal Med.* (2009) 14:8–13. doi: 10.1016/j.siny.2008.08.010
27. Snoek KG, Peters NCJ, van Rosmalen J, van Heijst AFJ, Eggink AJ, Sikkel E, et al. The validity of the observed-to-expected lung-to-head ratio in congenital diaphragmatic hernia in an era of standardized neonatal treatment; a multicenter study. *Prenatal Diagnosis.* (2017) 37:658–65. doi: 10.1002/pd.5062
28. Ferguson DM, Gupta VS, Lally PA, Luco M, Tsao K, Lally KP, et al. Postnatal pulmonary hypertension severity predicts inpatient outcomes in congenital diaphragmatic hernia. *Neonatology.* (2021) 118:147–54. doi: 10.1159/000512966
29. Brindle ME, Cook EF, Tibboel D, Lally PA, Lally KP. A clinical prediction rule for the severity of congenital diaphragmatic hernias in newborns. *Pediatrics.* (2014) 134:e413–9. doi: 10.1542/peds.2013-3367
30. Yu L, Sawle AD, Wynn J, Aspelund G, Stolar CJ, Arkovitz MS, et al. Increased burden of *de novo* predicted deleterious variants in complex congenital diaphragmatic hernia. *Human Mol Genet.* (2015) 24:4764–73. doi: 10.1093/hmg/ddv196
31. Cochius-den Otter SCM, Erdem Ö, van Rosmalen J, Schaible T, Peters NCJ, Cohen-Overbeek TE, et al. Validation of a prediction rule for mortality in congenital diaphragmatic hernia. *Pediatrics.* (2020) 145:e20192379. doi: 10.1542/peds.2019-2379
32. Stoll C, Alembik Y, Dott B, Roth MP. Associated malformations in cases with congenital diaphragmatic hernia. *Genet Couns.* (2008) 19:331–9.
33. Zaiss I, Kehl S, Link K, Neff W, Schaible T, Sütterlin M, et al. Associated malformations in congenital diaphragmatic hernia. *Am J Perinatol.* (2011) 28:211–8. doi: 10.1055/s-0030-1268235
34. Qiao L, Wynn J, Yu L, Hernan R, Zhou X, Duron V, et al. Likely damaging *de novo* variants in congenital diaphragmatic hernia patients are associated with worse clinical outcomes. *Genet Med.* (2020) 22:2020–8. doi: 10.1038/s41436-020-0908-0
35. Longoni M, High FA, Russell MK, Kashani A, Tracy AA, Coletti CM, et al. Molecular pathogenesis of congenital diaphragmatic hernia revealed by exome sequencing, developmental data, and bioinformatics. *Proc Natl Acad Sci USA.* (2014) 111:12450–5. doi: 10.1073/pnas.1412509111
36. Bendixen C, Reutter H. The role of *de novo* variants in patients with congenital diaphragmatic hernia. *Genes.* (2021) 12:1405. doi: 10.3390/genes12091405
37. Amoils M, Crisham Janik M, Lustig LR. Patterns and predictors of sensorineural hearing loss in children with congenital diaphragmatic hernia. *JAMA Otolaryngol.* (2015) 141:923–6. doi: 10.1001/jamaoto.2015.1670
38. Masumoto K, Nagata K, Uesugi T, Yamada T, Taguchi T. Risk factors for sensorineural hearing loss in survivors with severe congenital diaphragmatic hernia. *Euro J Pediatr.* (2007) 166:607–12. doi: 10.1007/s00431-006-0300-3
39. Coughlin MA, Werner NL, Gajarski R, Gadepalli S, Hirschl R, Barks J, et al. Prenatally diagnosed severe CDH: mortality and morbidity remain high. *J Pediatric Surg.* (2016) 51:1091–5. doi: 10.1016/j.jpedsurg.2015.10.082
40. Coughlin MA, Gupta VS, Ebanks AH, Harting MT, Lally KP. Incidence and outcomes of patients with congenital diaphragmatic hernia and pulmonary sequestration. *J Pediatric Surg.* (2021) 56:1126–9. doi: 10.1016/j.jpedsurg.2021.02.032
41. Lane KB, Machado RD, Pauciulo MW, Thomson JR, Phillips JA, 3rd, Loyd JE, et al. Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. *Nat Genet.* (2000) 26:81–4. doi: 10.1038/79226
42. Thomson JR, Machado RD, Pauciulo MW, Morgan NV, Humbert M, Elliott GC, et al. Sporadic primary pulmonary hypertension is associated with germline mutations of the gene encoding BMPR-II, a receptor member of the TGF-β family. *J Med Genet.* (2000) 37:741–5. doi: 10.1136/jmg.37.10.741
43. Nasim MT, Ogo T, Ahmed M, Randall R, Chowdhury HM, Snape KM, et al. Molecular genetic characterization of SMAD signaling molecules in pulmonary arterial hypertension. *Human Mutation.* (2011) 32:1385–9. doi: 10.1002/humu.21605
44. Shintani M, Yagi H, Nakayama T, Saji T, Matsuoka R. A new nonsense mutation of *SMAD8* associated with pulmonary arterial hypertension. *J Med Genet.* (2009) 46:331–7. doi: 10.1136/jmg.2008.062703
45. Zhu N, Gonzaga-Jauregui C, Welch CL, Ma L, Qi H, King AK, et al. Exome sequencing in children with pulmonary arterial hypertension demonstrates differences compared with adults. *Circul Genomic Precision Med.* (2018) 11:e001887. doi: 10.1161/CIRCGEN.117.001887
46. Khoshgoo N, Kholdebarin R, Pereira-Terra P, Mahood TH, Falk L, Day CA, et al. Prenatal microRNA miR-200b therapy improves nitrofen-induced pulmonary hypoplasia associated with congenital diaphragmatic hernia. *Ann Surg.* (2019) 269:979–87. doi: 10.1097/SLA.0000000000002595
47. Pereira-Terra P, Deprest JA, Kholdebarin R, Khoshgoo N, DeKoninck P, Munck AA, et al. Unique tracheal fluid microrna signature predicts response to FETO in patients with congenital diaphragmatic hernia. *Ann Surg.* (2015) 262:1130–40. doi: 10.1097/SLA.0000000000001054

48. MacFarlane EG, Haupt J, Dietz HC, Shore EM. TGF-β family signaling in connective tissue and skeletal diseases. *Cold Spring Harbor Perspect Biol.* (2017) 9:a022269. doi: 10.1101/cshperspect.a022269
49. Jay PY, Bielinska M, Erlich JM, Mannisto S, Pu WT, Heikinheimo M, et al. Impaired mesenchymal cell function in Gata4 mutant mice leads to diaphragmatic hernias and primary lung defects. *Dev Biol.* (2007) 301:602–14. doi: 10.1016/j.ydbio.2006.09.050
50. Yu L, Wynn J, Cheung YH, Shen Y, Mychaliska GB, Crombleholme TM, et al. Variants in GATA4 are a rare cause of familial and sporadic congenital diaphragmatic hernia. *Human Genet.* (2013) 132:285–92. doi: 10.1007/s00439-012-1249-0
51. Donahoe PK, Longoni M, High FA. Polygenic causes of congenital diaphragmatic hernia produce common lung pathologies. *Am J Pathol.* (2016) 186:2532–43. doi: 10.1016/j.ajpath.2016.07.006
52. Brady PD, Van Houdt J, Callewaert B, Deprest J, Devriendt K, Vermeesch JR. Exome sequencing identifies ZFPM2 as a cause of familial isolated congenital diaphragmatic hernia and possibly cardiovascular malformations. *Euro J Med Genet.* (2014) 57:247–52. doi: 10.1016/j.ejmg.2014.04.006
53. Beck TF, Campeau PM, Jhangiani SN, Gambin T, Li AH, Abo-Zahrah R, et al. FBN1 contributing to familial congenital diaphragmatic hernia. *Am J Med Genet A*. (2015) 167a:831–6. doi: 10.1002/ajmg.a.36960
54. Lobaton GO, Chen YJ, Jelin E, Garcia AV. Unusual case of delayed congenital diaphragmatic hernia in Loeys-Dietz syndrome: a case report. *J Surg Case Rep.* (2021) 2021:rjaa604. doi: 10.1093/jscr/rjaa604
55. Marsili L, Overwater E, Hanna N, Baujat G, Baars MJH, Boileau C, et al. Phenotypic spectrum of TGFB3 disease-causing variants in a Dutch-French cohort and first report of a homozygous patient. *Clin Genet.* (2020) 97:723–30. doi: 10.1111/cge.13700
56. Zaidi SH, Meyer S, Peltekova I, Teebi AS, Faiyaz-Ul-Haque M. Congenital diaphragmatic abnormalities in arterial tortuosity syndrome patients who carry mutations in the SLC2A10 gene. *Clin Genet.* (2009) 75:588–9. doi: 10.1111/j.1399-0004.2009.01165.x
57. MacCarrick G, Black JH, Bowdin S, El-Hamamsy I, Frischmeyer-Guerrerio PA, Guerrerio AL, et al. Loeys–Dietz syndrome: a primer for diagnosis and management. *Genet Med.* (2014) 16:576–87. doi: 10.1038/gim.2014.11
58. Yetman AT, Bornemeier RA, McCrindle BW. Long-term outcome in patients with Marfan syndrome: is aortic dissection the only cause of sudden death? *J Am Coll Cardiol.* (2003) 41:329–32. doi: 10.1016/S0735-1097(02)02699-2
59. Clugston RD, Zhang W, Alvarez S, de Lera AR, Greer JJ. Understanding abnormal retinoid signaling as a causative mechanism in congenital diaphragmatic hernia. *Am J Respir Cell Mol Biol.* (2010) 42:276–85. doi: 10.1165/rcmb.2009-0076OC
60. Dillon E, Renwick M, Wright C. Congenital diaphragmatic herniation: antenatal detection and outcome. *Br J Radiol.* (2000) 73:360–5. doi: 10.1259/bjr.73.868.10844860
61. Wolstenholme J, Brown J, Masters KG, Wright C, English CJ. Blepharophimosis sequence and diaphragmatic hernia associated with interstitial deletion of chromosome 3 (46,XY,del(3)(q21q23)). *J Med Genet.* (1994) 31:647–8. doi: 10.1136/jmg.31.8.647
62. Srour M, Chitayat D, Caron V, Chassaing N, Bitoun P, Patry L, et al. Recessive and dominant mutations in retinoic acid receptor beta in cases with microphthalmia and diaphragmatic hernia. *Am J Hum Genet.* (2013) 93:765–72. doi: 10.1016/j.ajhg.2013.08.014
63. Pasutto F, Sticht H, Hammersen G, Gillessen-Kaesbach G, Fitzpatrick DR, Nürnberg G, et al. Mutations in STRA6 cause a broad spectrum of malformations including anophthalmia, congenital heart defects, diaphragmatic hernia, alveolar capillary dysplasia, lung hypoplasia, mental retardation. *Am J Hum Genet.* (2007) 80:550–60. doi: 10.1086/512203
64. Kantarci S, Al-Gazali L, Hill RS, Donnai D, Black GC, Bieth E, et al. Mutations in LRP2, which encodes the multiligand receptor megalin, cause Donnai-Barrow and facio-oculo-acoustico-renal syndromes. *Nat Genet.* (2007) 39:957–9. doi: 10.1038/ng2063
65. Kammoun M, Brady P, De Catte L, Deprest J, Devriendt K, Vermeesch JR. Congenital diaphragmatic hernia as a part of Nance-Horan syndrome? *Euro J Human Genet.* (2018) 26:359–66. doi: 10.1038/s41431-017-0032-z
66. Mesas Burgos C, Ehrén H, Conner P, Frenckner B. Maternal risk factors and perinatal characteristics in congenital diaphragmatic hernia: a nationwide population-based study. *Fetal Diagnosis Ther.* (2019) 46:385–91. doi: 10.1159/000497619
67. Blomberg MI, Källén B. Maternal obesity and morbid obesity: the risk for birth defects in the offspring. *Birth Defects Res.* (2010) 88:35–40. doi: 10.1002/bdra.20620
68. Waller DK, Shaw GM, Rasmussen SA, Hobbs CA, Canfield MA, Siega-Riz AM, et al. Prepregnancy obesity as a risk factor for structural birth defects. *Arch Pediatr Adolescent Med.* (2007) 161:745–50. doi: 10.1001/archpedi.161.8.745
69. McAteer JP, Hecht A, De Roos AJ, Goldin AB. Maternal medical and behavioral risk factors for congenital diaphragmatic hernia. *J Pediatric Surg.* (2014) 49:34–8; discussion: 38. doi: 10.1016/j.jpedsurg.2013.09.025
70. Anderson KN, Lind JN, Simeone RM, Bobo WV, Mitchell AA, Riehle-Colarusso T, et al. Maternal use of specific antidepressant medications during early pregnancy and the risk of selected birth defects. *JAMA Psychiatry.* (2020) 77:1246–55. doi: 10.1001/jamapsychiatry.2020.2453
71. Crider KS, Cleves MA, Reefhuis J, Berry RJ, Hobbs CA, Hu DJ. Antibacterial medication use during pregnancy and risk of birth defects: National Birth Defects Prevention Study. *Arch Pediatr Adolescent Med.* (2009) 163:978–85. doi: 10.1001/archpediatrics.2009.188
72. Carter TC, Druschel CM, Romitti PA, Bell EM, Werler MM, Mitchell AA. Antifungal drugs and the risk of selected birth defects. *Am J Obstetr Gynecol.* (2008) 198:191.e1–7. doi: 10.1016/j.ajog.2007.08.044
73. Perez-Aytes A, Marin-Reina P, Boso V, Ledo A, Carey JC, Vento M. Mycophenolate mofetil embryopathy: a newly recognized teratogenic syndrome. *Euro J Med Genet.* (2017) 60:16–21. doi: 10.1016/j.ejmg.2016.09.014
74. Dawson AL, Riehle-Colarusso T, Reefhuis J, Arena JF. Maternal exposure to methotrexate and birth defects: a population-based study. *Am J Med Genet A.* (2014) 164a:2212–6. doi: 10.1002/ajmg.a.36625
75. Ramakrishnan R, Stuart AL, Salemi JL, Chen H, O'Rourke K, Kirby RS. Maternal exposure to ambient cadmium levels, maternal smoking during pregnancy, and congenital diaphragmatic hernia. *Birth Defects Res.* (2019) 111:1399–407. doi: 10.1002/bdr2.1555
76. Carmichael SL, Yang W, Roberts E, Kegley SE, Brown TJ, English PB, et al. Residential agricultural pesticide exposures and risks of selected birth defects among offspring in the San Joaquin Valley of California. *Birth Defects Res.* (2016) 106:27–35. doi: 10.1002/bdra.23459
77. Schulz F, Jenetzky E, Zwink N, Bendixen C, Kipfmueller F, Rafat N, et al. Parental risk factors for congenital diaphragmatic hernia - a large German case-control study. *BMC Pediatr.* (2021) 21:278. doi: 10.1186/s12887-021-02748-3
78. Balayla J, Abenhaim HA. Incidence, predictors and outcomes of congenital diaphragmatic hernia: a population-based study of 32 million births in the United States. *J Maternal Fetal Neonatal Med.* (2014) 27:1438–44. doi: 10.3109/14767058.2013.858691
79. Felix JF, van Dooren MF, Klaassens M, Hop WC, Torfs CP, Tibboel D. Environmental factors in the etiology of esophageal atresia and congenital diaphragmatic hernia: results of a case-control study. *Birth Defects Res.* (2008) 82:98–105. doi: 10.1002/bdra.20423
80. Caspers KM, Oltean C, Romitti PA, Sun L, Pober BR, Rasmussen SA, et al. Maternal periconceptional exposure to cigarette smoking and alcohol consumption and congenital diaphragmatic hernia. *Birth Defects Res.* (2010) 88:1040–9. doi: 10.1002/bdra.20716
81. Yang W, Shaw GM, Carmichael SL, Rasmussen SA, Waller DK, Pober BR, et al. Nutrient intakes in women and congenital diaphragmatic hernia in their offspring. *Birth Defects Res.* (2008) 82:131–8. doi: 10.1002/bdra.20436
82. Carmichael SL, Ma C, Witte JS, Yang W, Rasmussen SA, Brunelli L, et al. Congenital diaphragmatic hernia and maternal dietary nutrient pathways and diet quality. *Birth Defects Res.* (2020) 112:1475–83. doi: 10.1002/bdr2.1770
83. Beurskens LW, Schrijver LH, Tibboel D, Wildhagen MF, Knapen MF, Lindemans J, et al. Dietary vitamin A intake below the recommended daily intake during pregnancy and the risk of congenital diaphragmatic hernia in the offspring. *Birth Defects Res.* (2013) 97:60–6. doi: 10.1002/bdra.23093
84. Michikawa T, Yamazaki S, Sekiyama M, Kuroda T, Nakayama SF, Isobe T, et al. Maternal dietary intake of vitamin A during pregnancy was inversely associated with congenital diaphragmatic hernia: the Japan

Environment and Children's Study. *Br J Nutr.* (2019) 122:1295–302. doi: 10.1017/S0007114519002204
85. Beurskens LW, Tibboel D, Lindemans J, Duvekot JJ, Cohen-Overbeek TE, Veenma DC, et al. Retinol status of newborn infants is associated with congenital diaphragmatic hernia. *Pediatrics.* (2010) 126:712–20. doi: 10.1542/peds.2010-0521
86. Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, et al. Epigenetic differences arise during the lifetime of monozygotic twins. *Proc Natl Acad Sci USA.* (2005) 102:10604–9. doi: 10.1073/pnas.0500398102
87. Malki K, Koritskaya E, Harris F, Bryson K, Herbster M, Tosto MG. Epigenetic differences in monozygotic twins discordant for major depressive disorder. *Transl Psychiatry.* (2016) 6:e839. doi: 10.1038/tp.2016.101
88. Castillo-Fernandez JE, Spector TD, Bell JT. Epigenetics of discordant monozygotic twins: implications for disease. *Genome Med.* (2014) 6:60. doi: 10.1186/s13073-014-0060-z
89. Jaffe AE, Irizarry RA. Accounting for cellular heterogeneity is critical in epigenome-wide association studies. *Genome Biol.* (2014) 15:R31. doi: 10.1186/gb-2014-15-2-r31
90. Gervin K, Salas LA, Bakulski KM, van Zelm MC, Koestler DC, Wiencke JK, et al. Systematic evaluation and validation of reference and library selection methods for deconvolution of cord blood DNA methylation data. *Clin Epigenet.* (2019) 11:125. doi: 10.1186/s13148-019-0717-y
91. Rahmani E, Schweiger R, Rhead B, Criswell LA, Barcellos LF, Eskin E, et al. Cell-type-specific resolution epigenetics without the need for cell sorting or single-cell biology. *Nat Commun.* (2019) 10:3417. doi: 10.1038/s41467-019-11052-9
92. Pober BR, Lin A, Russell M, Ackerman KG, Chakravorty S, Strauss B, et al. Infants with Bochdalek diaphragmatic hernia: sibling precurrence and monozygotic twin discordance in a hospital-based malformation surveillance program. *Am J Med Genet.* (2005) 138A:81–8. doi: 10.1002/ajmg.a.30904
93. Wang W, Pan W, Chen J, Xie W, Liu M, Wang J. Outcomes of congenital diaphragmatic hernia in one of the twins. *Am J Perinatol.* (2019) 36:1304–9. doi: 10.1055/s-0038-1676830
94. Veenma D, Brosens E, de Jong E, van de Ven C, Meeussen C, Cohen-Overbeek T, et al. Copy number detection in discordant monozygotic twins of Congenital Diaphragmatic Hernia (CDH) and Esophageal Atresia (EA) cohorts. *Euro J Human Genet.* (2012) 20:298–304. doi: 10.1038/ejhg.2011.194
95. MacKenzie KC, Garritsen R, Chauhan RK, Sribudiani Y, de Graaf BM, Rugenbrink T, et al. The somatic mutation paradigm in congenital malformations: hirschsprung disease as a model. *Int J Mol Sci.* (2021) 22:12354. doi: 10.3390/ijms222212354
96. Matsunami N, Shanmugam H, Baird L, Stevens J, Byrne JL, Barnhart DC, et al. Germline but not somatic *de novo* mutations are common in human congenital diaphragmatic hernia. *Birth Defects Res.* (2018) 110:610–7. doi: 10.1002/bdr2.1223
97. Bogenschutz EL, Fox ZD, Farrell A, Wynn J, Moore B, Yu L, et al. Deep whole-genome sequencing of multiple proband tissues and parental blood reveals the complex genetic etiology of congenital diaphragmatic hernias. *HGG Adv.* (2020) 1:100008. doi: 10.1101/2020.04.03.024398
98. Qi H, Yu L, Zhou X, Wynn J, Zhao H, Guo Y, et al. *De novo* variants in congenital diaphragmatic hernia identify MYRF as a new syndrome and reveal genetic overlaps with other developmental disorders. *PLoS Genet.* (2018) 14:e1007822. doi: 10.1371/journal.pgen.1007822
99. Devriendt K, Deloof E, Moerman P, Legius E, Vanhole C, de Zegher F, et al. Diaphragmatic hernia in Denys-Drash syndrome. *Am J Med Genet.* (1995) 57:97–101. doi: 10.1002/ajmg.1320570120
100. Antonius T, van Bon B, Eggink A, van der Burgt I, Noordam K, van Heijst A. Denys-Drash syndrome and congenital diaphragmatic hernia: another case with the 1097G > A(Arg366His) mutation. *Am J Med Genet A.* (2008) 146a:496–9. doi: 10.1002/ajmg.a.32168
101. Suri M, Kelehan P, O'Neill D, Vadeyar S, Grant J, Ahmed SF, et al. WT1 mutations in Meacham syndrome suggest a coelomic mesothelial origin of the cardiac and diaphragmatic malformations. *Am J Med Genet.* (2007) 143a:2312–20. doi: 10.1002/ajmg.a.31924
102. Denamur E, Bocquet N, Baudouin V, Da Silva F, Veitia R, Peuchmaur M, et al. WT1 splice-site mutations are rarely associated with primary steroid-resistant focal and segmental glomerulosclerosis. *Kidney Int.* (2000) 57:1868–72. doi: 10.1046/j.1523-1755.2000.00036.x
103. Schwab ME, Dong S, Lianoglou BR, Aguilar Lucero AF, Schwartz GB, Norton ME, et al. Exome sequencing of fetuses with congenital diaphragmatic hernia supports a causal role for NR2F2, PTPN11, WT1 variants. *Am J Surg.* (2021). doi: 10.1016/j.amjsurg.2021.07.016
104. Qiao L, Xu L, Yu L, Wynn J, Hernan R, Zhou X, et al. Rare and *de novo* variants in 827 congenital diaphragmatic hernia probands implicate LONP1 as candidate risk gene. *Am J Hum Genet.* (2021) 108:1964–80. doi: 10.1016/j.ajhg.2021.08.011
105. Scott TM, Campbell IM, Hernandez-Garcia A, Lalani SR, Liu P, Shaw CA, et al. Clinical exome sequencing data reveal high diagnostic yields for congenital diaphragmatic hernia plus (CDH+) and new phenotypic expansions involving CDH. *J Med Genet.* (2021). doi: 10.1136/jmedgenet-2020-107317. [Epub ahead of print].
106. Jacobs AM, Toudjarska I, Racine A, Tsipouras P, Kilpatrick MW, Shanske A. A recurring FBN1 gene mutation in neonatal Marfan syndrome. *Arch Pediatr Adolesc Med.* (2002) 156:1081–5. doi: 10.1001/archpedi.156.11.1081
107. Revencu N, Quenum G, Detaille T, Verellen G, De Paepe A, Verellen-Dumoulin C. Congenital diaphragmatic eventration and bilateral uretero-hydronephrosis in a patient with neonatal Marfan syndrome caused by a mutation in exon 25 of the FBN1 gene and review of the literature. *Euro J Pediatr.* (2004) 163:33–7. doi: 10.1007/s00431-003-1330-8
108. Stheneur C, Faivre L, Collod-Béroud G, Gautier E, Binquet C, Bonithon-Kopp C, et al. Prognosis factors in probands with an FBN1 mutation diagnosed before the age of 1 year. *Pediatric Res.* (2011) 69:265–70. doi: 10.1203/PDR.0b013e3182097219
109. Hosokawa S, Takahashi N, Kitajima H, Nakayama M, Kosaki K, Okamoto N. Brachmann-de Lange syndrome with congenital diaphragmatic hernia and NIPBL gene mutation. *Congenital Anomalies.* (2010) 50:129–32. doi: 10.1111/j.1741-4520.2010.00270.x
110. Donnai D, Barrow M. Diaphragmatic hernia, exomphalos, absent corpus callosum, hypertelorism, myopia, and sensorineural deafness: a newly recognized autosomal recessive disorder? *Am J Med Genet.* (1993) 47:679–82. doi: 10.1002/ajmg.1320470518
111. Maas SM, Lombardi MP, van Essen AJ, Wakeling EL, Castle B, Temple IK, et al. Phenotype and genotype in 17 patients with Goltz-Gorlin syndrome. *J Med Genet.* (2009) 46:716–20. doi: 10.1136/jmg.2009.068403
112. Yano S, Baskin B, Bagheri A, Watanabe Y, Moseley K, Nishimura A, et al. Familial Simpson-Golabi-Behmel syndrome: studies of X-chromosome inactivation and clinical phenotypes in two female individuals with GPC3 mutations. *Clin Genet.* (2011) 80:466–71. doi: 10.1111/j.1399-0004.2010.01554.x
113. Hogue J, Shankar S, Perry H, Patel R, Vargervik K, Slavotinek A. A novel EFNB1 mutation (c.712delG) in a family with craniofrontonasal syndrome and diaphragmatic hernia. *Am J Med Genet A.* (2010) 152a:2574–7. doi: 10.1002/ajmg.a.33596
114. Yu L, Hernan RR, Wynn J, Chung WK. The influence of genetics in congenital diaphragmatic hernia. *Semin Perinatol.* (2020) 44:151169. doi: 10.1053/j.semperi.2019.07.008
115. Klaassens M, Scott DA, van Dooren M, Hochstenbach R, Eussen HJ, Cai WW, et al. Congenital diaphragmatic hernia associated with duplication of 11q23-qter. *Am J Med Genet A.* (2006) 140:1580–6. doi: 10.1002/ajmg.a.31321
116. Goumy C, Laffargue F, Eymard-Pierre E, Kemeny S, Gay-Bellile M, Gouas L, et al. Congenital diaphragmatic hernia may be associated with 17q12 microdeletion syndrome. *Am J Med Genet A.* (2015) 167a:250–3. doi: 10.1002/ajmg.a.36840
117. Hendrix NW, Clemens M, Canavan TP, Surti U, Rajkovic A. Prenatally diagnosed 17q12 microdeletion syndrome with a novel association with congenital diaphragmatic hernia. *Fetal Diagnosis Ther.* (2012) 31:129–33. doi: 10.1159/000332968
118. Wat MJ, Shchelochkov OA, Holder AM, Breman AM, Dagli A, Bacino C, et al. Chromosome 8p23.1 deletions as a cause of complex congenital heart defects and diaphragmatic hernia. *Am J Med Genet A.* (2009) 149a:1661–77. doi: 10.1002/ajmg.a.32896
119. Longoni M, Lage K, Russell MK, Loscertales M, Abdul-Rahman OA, Baynam G, et al. Congenital diaphragmatic hernia interval on chromosome 8p23.1

characterized by genetics and protein interaction networks. *Am J Med Genet A*. (2012) 158a:3148–58. doi: 10.1002/ajmg.a.35665
120. Slavotinek A, Lee SS, Davis R, Shrit A, Leppig KA, Rhim J, et al. Fryns syndrome phenotype caused by chromosome microdeletions at 15q26.2 and 8p23.1. *J Med Genet.* (2005) 42:730–6. doi: 10.1136/jmg.2004.028787
121. Wat MJ, Beck TF, Hernández-García A, Yu Z, Veenma D, Garcia M, et al. Mouse model reveals the role of SOX7 in the development of congenital diaphragmatic hernia associated with recurrent deletions of 8p23.1. *Human Mol Genet.* (2012) 21:4115–25. doi: 10.1093/hmg/dds241
122. Longoni M, Russell MK, High FA, Darvishi K, Maalouf FI, Kashani A, et al. Prevalence and penetrance of ZFPM2 mutations and deletions causing congenital diaphragmatic hernia. *Clin Genet.* (2015) 87:362–7. doi: 10.1111/cge.12395
123. Klaassens M, van Dooren M, Eussen HJ, Douben H, den Dekker AT, Lee C, et al. Congenital diaphragmatic hernia and chromosome 15q26: determination of a candidate region by use of fluorescent in situ hybridization and array-based comparative genomic hybridization. *Am J Hum Genet.* (2005) 76:877–82. doi: 10.1086/429842
124. High FA, Bhayani P, Wilson JM, Bult CJ, Donahoe PK, Longoni M. *De novo* frameshift mutation in COUP-TFII (NR2F2) in human congenital diaphragmatic hernia. *Am J Med Genet A*. (2016) 170:2457–61. doi: 10.1002/ajmg.a.37830
125. Kantarci S, Casavant D, Prada C, Russell M, Byrne J, Haug LW, et al. Findings from aCGH in patients with congenital diaphragmatic hernia (CDH): a possible locus for Fryns syndrome. *Am J Med Genet A*. (2006) 140:17–23. doi: 10.1002/ajmg.a.31025
126. Slavotinek AM, Moshrefi A, Lopez Jiminez N, Chao R, Mendell A, Shaw GM, et al. Sequence variants in the HLX gene at chromosome 1q41-1q42 in patients with diaphragmatic hernia. *Clin Genet.* (2009) 75:429–39. doi: 10.1111/j.1399-0004.2009.01182.x
127. Shaffer LG, Theisen A, Bejjani BA, Ballif BC, Aylsworth AS, Lim C, et al. The discovery of microdeletion syndromes in the post-genomic era: review of the methodology and characterization of a new 1q41q42 microdeletion syndrome. *Genet Med.* (2007) 9:607–16. doi: 10.1097/GIM.0b013e3181484b49
128. Slavotinek AM, Moshrefi A, Davis R, Leeth E, Schaeffer GB, Burchard GE, et al. Array comparative genomic hybridization in patients with congenital diaphragmatic hernia: mapping of four CDH-critical regions and sequencing of candidate genes at 15q26.1-15q26.2. *Euro J Human Genet.* (2006) 14:999–1008. doi: 10.1038/sj.ejhg.5201652
129. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* (2015) 17:405–24. doi: 10.1038/gim.2015.30
130. Longoni M, High FA, Qi H, Joy MP, Hila R, Coletti CM, et al. Genome-wide enrichment of damaging *de novo* variants in patients with isolated and complex congenital diaphragmatic hernia. *Human Genet.* (2017) 136:679–91. doi: 10.1007/s00439-017-1774-y
131. Merrell AJ, Ellis BJ, Fox ZD, Lawson JA, Weiss JA, Kardon G. Muscle connective tissue controls development of the diaphragm and is a source of congenital diaphragmatic hernias. *Nat Genet.* (2015) 47:496–504. doi: 10.1038/ng.3250
132. Paris ND, Coles GL, Ackerman KG. Wt1 and β-catenin cooperatively regulate diaphragm development in the mouse. *Dev Biol.* (2015) 407:40–56. doi: 10.1016/j.ydbio.2015.08.009
133. Coles GL, Ackerman KG. Kif7 is required for the patterning and differentiation of the diaphragm in a model of syndromic congenital diaphragmatic hernia. *Proc Natl Acad Sci USA*. (2013) 110:E1898–905. doi: 10.1073/pnas.1222797110
134. Clugston RD, Zhang W, Greer JJ. Early development of the primordial mammalian diaphragm and cellular mechanisms of nitrofen-induced congenital diaphragmatic hernia. *Birth Defects Res.* (2010) 88:15–24. doi: 10.1002/bdra.20613
135. Beurskens N, Klaassens M, Rottier R, de Klein A, Tibboel D. Linking animal models to human congenital diaphragmatic hernia. *Birth Defects Res.* (2007) 79:565–72. doi: 10.1002/bdra.20370
136. Nakamura H, Doi T, Puri P, Friedmacher F. Transgenic animal models of congenital diaphragmatic hernia: a comprehensive overview of candidate genes and signaling pathways. *Pediatric Surg Int.* (2020) 36:991–7. doi: 10.1007/s00383-020-04705-0
137. Hornstra IK, Birge S, Starcher B, Bailey AJ, Mecham RP, Shapiro SD. Lysyl oxidase is required for vascular and diaphragmatic development in mice. *J Biol Chem.* (2003) 278:14387–93. doi: 10.1074/jbc.M210144200
138. Noda K, Kitagawa K, Miki T, Horiguchi M, Akama TO, Taniguchi T, et al. A matricellular protein fibulin-4 is essential for the activation of lysyl oxidase. *Sci Adv.* (2020) 6:eabc1404. doi: 10.1126/sciadv.abc1404
139. Russell MK, Longoni M, Wells J, Maalouf FI, Tracy AA, Loscertales M, et al. Congenital diaphragmatic hernia candidate genes derived from embryonic transcriptomes. *Proc Natl Acad Sci USA*. (2012) 109:2978–83. doi: 10.1073/pnas.1121621109
140. Kruse SW, Suino-Powell K, Zhou XE, Kretschman JE, Reynolds R, Vonrhein C, et al. Identification of COUP-TFII orphan nuclear receptor as a retinoic acid-activated receptor. *PLoS Biol.* (2008) 6:e227. doi: 10.1371/journal.pbio.0060227
141. Engelen E, Akinci U, Bryne JC, Hou J, Gontan C, Moen M, et al. Sox2 cooperates with Chd7 to regulate genes that are mutated in human syndromes. *Nat Genet.* (2011) 43:607–11. doi: 10.1038/ng.825
142. Beck TF, Veenma D, Shchelochkov OA, Yu Z, Kim BJ, Zaveri HP, et al. Deficiency of FRAS1-related extracellular matrix 1 (FREM1) causes congenital diaphragmatic hernia in humans and mice. *Human Mol Genet.* (2012) 22:1026–38. doi: 10.1093/hmg/dds507
143. Reiss I, Schaible T, van den Hout L, Capolupo I, Allegaert K, van Heijst A, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH EURO Consortium consensus. *Neonatology.* (2010) 98:354–64. doi: 10.1159/000320622
144. Congenital Diaphragmatic Hernia Study Group. Estimating disease severity of congenital diaphragmatic hernia in the first 5 minutes of life. *J Pediatric Surg.* (2001) 36:141–5. doi: 10.1053/jpsu.2001.20032
145. Bonomi L, Huang Y, Ohno-Machado L. Privacy challenges and research opportunities for genomic data sharing. *Nat Genet.* (2020) 52:646–54. doi: 10.1038/s41588-020-0651-0
146. Byrd JB, Greene AC, Prasad DV, Jiang X, Greene CS. Responsible, practical genomic data sharing that accelerates research. *Nat Rev Genet.* (2020) 21:615–29. doi: 10.1038/s41576-020-0257-5

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Recurrence of Congenital Diaphragmatic Hernia: Risk Factors, Management, and Future Perspectives

Francesco Macchini[1†], Genny Raffaeli[2,3†], Ilaria Amodeo[2], Martina Ichino[1], José Luis Encinas[4], Leopoldo Martinez[4], Lucas Wessel[5] and Giacomo Cavallaro[2]*

[1] Department of Pediatric Surgery, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, [2] Neonatal Intensive Care Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy, [3] Department of Clinical Sciences and Community Health, Università degli Studi di Milano, Milan, Italy, [4] Department of Pediatric Surgery, La Paz Children's Hospital, Universidad Autónoma de Madrid, Madrid, Spain, [5] Department of Pediatric Surgery, Faculty of Medicine Mannheim at Heidelberg University, Mannheim University Medical Center, Mannheim, Germany

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Edited by:
Neil Patel,
Royal Hospital for Children,
United Kingdom

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Carl Davis,
Royal Hospital for Children,
United Kingdom

***Correspondence:**
Giacomo Cavallaro
giacomo.cavallaro@policlinico.mi.it

[†] These authors share first authorship

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Recurrence is one of the most common surgical complications in Congenital Diaphragmatic Hernia (CDH). It could remain clinically silent for a long time or present as an acute complication week, months, or even years after the primary surgery. Several risk factors have been identified so far. An extended diaphragmatic defect represents one of the leading independent risk factors, together with indirect signs of large defect such as the liver position related to the diaphragm and the use of the prosthetic patch and with the use of a minimally invasive surgical (MIS) approach. However, the exact contribution of each factor and the overall risk of recurrence during the life span still need to be fully understood. This mini-review aims to give an overview of the current knowledge regarding CDH recurrence, focusing on predisposing factors, clinical presentation, management and follow-up of high-risk patients, and future perspectives.

Keywords: congenital diaphragmatic hernia, hernia recurrence, minimally invasive surgery, pulmonary hypertension, mortality, prosthetic patch, FETO

INTRODUCTION

Recurrence of Congenital Diaphragmatic Hernia (CDH) represents a common complication in CDH survivors, along with pulmonary, gastrointestinal, neurobehavioral, and developmental anomalies (1–4). It mostly happens at the site of the original hernia, but occasionally hiatal hernia may follow CDH repair due to tension on the diaphragmatic crura. Therefore, we will concentrate on this review over the first entity.

The incidence of recurrence after CDH repair varies considerably, ranging from 5 to 65% in reports with different lengths of follow-up and different follow-up protocols (4–11). The average age at recurrence is 12 months, with 47.6% of cases occurring before 1 year of age, 76.2% before 2 years, and near 100% before 5 years (12–14). Only 3% of cases are reported as an early in-hospital recurrence (2). In older children, the recurrences are rare (15).

PREDISPOSING FACTORS

Many different predisposing factors (PF) have been investigated related to pre- and postnatal life, congenital and acquired diseases, medical and surgical problems, with inconclusive results in different series.

Prenatal

Although there are authors that did not evidence differences in recurrence rate among prenatal patient-related characteristics (16), most studies report a higher recurrence rate in patients with signs likely related to a larger defect size such as lower observed/expected lung to head ratio (O/E LHR%), prenatal diagnosis of CDH (<22 weeks of gestational age), observed/expected total fetal lung volume (O/E TFLV) < 30%, thoracic position of the liver (5, 8, 10, 17, 18). A recent study by Amodeo et al. showed that patients prone to recurrence have lower final O/E LHR% during fetal life and could be identified in the early postnatal life by estimating the pulmonary surface at the first Chest X-ray (CXR) control after birth. Indeed, the unit increase in total and ipsilateral area in cm^2 was associated with a 14 and 29% reduction in the risk of recurrence, respectively (17). These findings further suggest that recurrence is related to the defect size. In addition, a large defect size has been associated with an early in-hospital recurrence (2). Another prenatal risk factor frequently reported in the literature is the absence of a hernia sac (5, 10, 18–20). There is still contrasting evidence concerning a higher recurrence risk in the right-sided defects (21, 22), while Fetal Endoscopic Tracheal Occlusion (FETO) procedure has not been confirmed as a predisposing factor for recurrence (2, 17, 21).

Postnatal

Many postnatal PFs seem to be associated with recurrence. Some might be indirect signs of larger defects, such as the need for ECMO and the use of diaphragmatic and abdominal patches. Others are generally related to the severity of the disease, such as prolonged invasive respiratory support, need for intensive care, longer duration of mechanical ventilation, post-operative sildenafil requirement, longer length of stay (LOS), age at discharge, supplemental oxygen requirement, persistent pulmonary hypertension. And others still, like thoracotomy and MIS, are related to surgical choice (12, 17, 23–25).

Surgical-related PFs seem to have a major role in recurrence among postnatal variables, especially the use of patches, both diaphragmatic and abdominal (16). Despite this, the use of patches for repair has been increasing in the last decade. Patients who require a diaphragmatic patch repair are reported to have a risk 2.83 times higher of developing a recurrence (26). The inability of the synthetic patch to grow with the patient is the mechanism underlying this strong association (2). But, again, the disease's severity and the defect's size may present an underlying independent role (26). The goal of the patch is to allow closure of the defect without tension on the surrounding structures, despite a large defect size, granting a tension-free suture. This aims to reduce the risk of recurrence and seems effective, as shown by Zahn et al. (27). Another advantage is the possibility to create an "over-sized" cone- or dome-shape for the new diaphragm, allowing for better respiratory physiology. A cone- or dome-shaped prosthetic patch gives the thoracic cavity a more physiologic shape and volume (**Figure 1**). Moreover, it provides additional abdominal volume during the significant growth of the first year of life, facilitating tissue ingrowth coming from folds of the redundant material sutured to the rims of the diaphragm (28). Nonetheless, some single-center studies do not report any significant difference in hernia recurrence rates between the patch and primary repair, while other authors even described a reduced recurrence rate in patients treated with a patch (6, 27, 29, 30), in contrast with data of large series from high-volume centers (6, 16, 26).

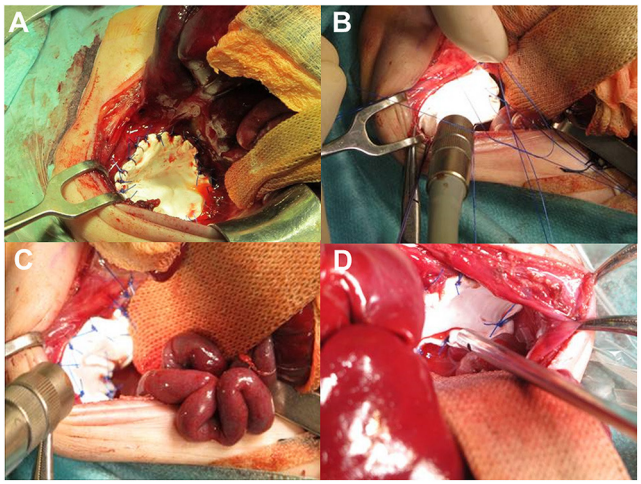

FIGURE 1 | Intraoperative imaging of patch repair. **(A,B)** Dome-shape patch repair. **(C,D)** Cone-shape patch repair.

Another open issue is the patch material. In a recent report, the non-absorbable polytetrafluoroethylene (PTFE) patch appears to have a lower recurrence rate than the absorbable intestinal submucosal (SIS) patch. This retrospective and monocentric study assessed the use of patches with a follow-up limited by the bias due to the sequentially timed implementation of the PTFE patch related to the SIS patch (31). Albeit the article by Camila et al. presents some limitations, the future seems promising for using PTFE patches (31).

Alternative methods for diaphragmatic breach closure have been suggested to avoid diaphragmatic patches, such as wall muscle flaps like the reversed latissimus dorsi muscle flap. This is suggested as an alternative to patches in case of significant defects or agenesis of the hemidiaphragm (28). Limited experiences have shown similar or even better outcomes with muscle flaps (32, 33). However, larger studies would be needed to confirm these results, and strong evidence in its favor is still failing. Moreover, the problem in muscle flaps is that innervation is missing, and we could see a marked dysfunction of diaphragm motility overall in massive C- and D-defects (34, 35).

Constant efforts are being made to find the "perfect" graft for diaphragmatic reconstruction, and the future of tissue-engineered diaphragmatic repair is promising (36).

Based on current evidence, major international study groups recommend using non-absorbable prosthetic patches, mainly PTFE, aiming at an oversized/dome shape. PTFE appears safe and is associated with a low recurrence rate (7, 10, 26, 37).

Another surgical PF is the use of an abdominal patch. Even if rapidly removed through staged closure, an abdominal prosthesis can predispose to recurrence by interfering with the integrity of the diaphragm at its connection to the anterior abdominal wall (16, 27).

Most surgeons agree that the recurrence rate also depends on the surgical technique (28). The postero-lateral section of the defect deserves particular attention and is deemed essential to secure the patch with particular care in this part of the defect, passing the stitches around the ribs and intercostal muscles, if necessary. Usually, a non-resorbable suture is used to secure the diaphragmatic patch (28). In addition, some technical expedients have been proposed to minimize the risk of recurrence. For instance, pledged sutures are used to strengthen the hold on the tissue or to tailor the patches in modified shapes such as double-layer patches (18).

The post-operative chest X-ray (CXR) may help evaluate the accuracy of surgery, and a flat-appearing diaphragm could be an indirect sign of a tense repair with a higher risk of recurrence. However, no relationship between post-operative CXR diaphragmatic appearance and recurrence has been observed (38).

Recently, minimally invasive surgery (MIS) has increased its pediatric and neonatal surgery applications, but CDH still represents a challenge for laparoscopic (anterior defects) and thoracoscopic (mostly Bochdalek defect) repairs. The advantages of MIS are mainly represented by less pain, less incisional complications, and reduced surgical stress compared to traditional surgery. In general, TR is not contraindicated in newborns since relative hypercapnia is tolerated (29). At the same time, thoracoscopic repair (TR) of CDH is reported to have a greater risk of recurrence (2–9%) than the classic repair through laparotomy (1–4%) (2). Cioci et al. also observed a significantly higher recurrence in those patients who underwent MIS repair (48%) as compared to open repair (OR) (16%) (23). However, significance was not reached in other series (30), and some recent studies have identified a similar risk of recurrence between TR and OR in selected patients (39–45).

Furthermore, the rate of recurrence in TR decreases with the increase of the surgeon's experience (learning curve) (2). Because the increased risk of recurrence with MIS repair would seem due to surgeon inexperience, several studies proposed that TR should be limited to high-volume centers and experienced surgeons (2). Nevertheless, other factors could be involved in the higher recurrence risk in MIS. Therefore, it has been suggested to limit MIS to the smallest defects, classified as A or B, by the Congenital Diaphragmatic Hernia Study Group (CDHSG) Staging System (23). Additional proposed selection criteria are cardiovascular stability and no pulmonary hypertension, mild symptoms or asymptomatic, liver down, late presentation or postnatal diagnosis, and absence of severe comorbidities (31, 46). However, further studies are needed, especially with a structured long-term follow-up.

At present, no correlation has been reported between time to surgery and risk of recurrence (16). The correlation between ECMO support and recurrence also requires some attention. The need for ECMO could independently increase the risk of recurrence or indicate a more severe clinical presentation with a larger defect size (2). Moreover, the recurrence rate is not associated with the repair timing (before, during, or after ECMO) and the need for the "EXIT to ECMO" procedure (2, 47, 48). However, these results are biased by the lack of standardized long-term follow-up in some series (2, 49).

A recent study observed the impact of hospital volume on CDH recurrence for the first time. Cioci et al. demonstrated as low-volume CDH centers have significantly higher recurrence rates and hospital costs than high-volume CDH hospitals. Therefore, the de-centralization of CDH patients would be a further PF (23). Consequently, through a hub and spoke model, the centralization of CDH delivery is needed to improve care and reduce costs, complications, morbidity, and mortality (50).

MANAGEMENT

The management is based on the severity of the condition. A minor recurrence is defined as a tiny defect in an asymptomatic patient, with minimal herniation of the abdominal content into the thorax, more frequently only the omentum, without worsening during follow-up. Recurrence is defined as major when it allows the stomach and/or bowel loops to re-herniate back up into the thorax or worsens over time (5, 6, 15).

In case of minor recurrences, conservative management may represent a good choice, avoiding re-operation, provided that the patient remains stable at periodic plain CXR and clinical examination for a minimum follow-up of 5 years (5, 6, 15, 27).

A surgical approach is indicated when a major recurrence is detected (**Figure 2**). At the dorsal costo-abdominal place, the sutures could grow through the ribs or could be torn out, leading to relatively small additional defects in the case of Bochdalek hernia. Nevertheless, a fault at the hiatus could be observed in other patients. Therefore, sometimes, an additional patch could be inserted without replacing the entire patch in both cases. Thoracotomy could be a good alternative in cases where the recurrence was located more ventral. Despite this, adhesions could even affect the thoracic cavity.

There is ultimately no consensus on the optimal surgical approach to CDH recurrence. Some authors suggest approaching a recurrence from a so-called "virgin plane", meaning the opposite body cavity compared to the first surgery (51). This aims to work in a more accessible surgical field with fewer adhesions and better visibility.

However, a recent survey shows that recurrence is repaired with the same technique (laparotomy, thoracotomy, MIS) as the primary operation in 48% of cases (23). In the open approach, laparotomy is always favored over thoracotomy. On the other hand, thoracoscopy is the preferred approach among MIS surgeons for the first surgery as well as recurrence, except in case of initial thoracotomy. Future prospective studies may help define the optimal approach. Still, in the absence of clear

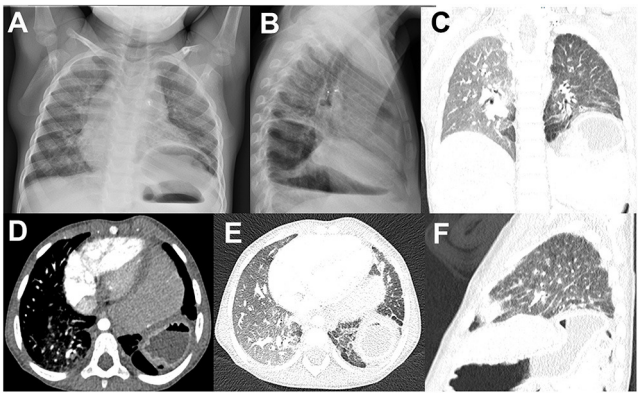

FIGURE 2 | Radiological image of the major recurrence of left side CDH after a first patched diaphragmatic closure. **(A,B)** Chest X-ray image, **(C–F)** computed tomography image.

evidence favoring a specific technique, it is preferable to use the most comfortable route to the operator.

RE-RECURRENCE

The incidence of a second recurrence after the first recurrence repair is not well documented in the literature, but it seems to be high, especially in D-defects. Moss et al. reported a second recurrence rate of 25% (52). In another series by Laituri et al., the frequency of a second recurrence was 50% among patients with CDH repaired with the patch (53). Another study showed a second recurrence rate of 19%, and re-repair was performed either by patch or by primary suturing (12).

Considering the risk of subsequent recurrences, a long-term multidisciplinary follow-up plays a key role in the timely detection of complications. Because re-re-operations are very demanding, a subtle technique in order to avoid further complications is needed. The previous patch can be left inside and a second patch added over it to reduce the risk of iatrogenic damage.

CLINICAL PRESENTATION AND FOLLOW-UP FOR TIMELY DIAGNOSIS OF HERNIA RECURRENCE

Clinical presentation of CDH recurrence may include dysphagia, retching, constipation, abdominal pain, failure to thrive, and progressive dyspnea up to respiratory failure. However, upper gastrointestinal symptoms should be carefully assessed to differentiate between reflux disease and possible hiatal hernia from recurrence. Sometimes, an acute bowel obstruction could be the presenting clinical picture of a misdiagnosed hernia recurrence. However, up to two-thirds of the patients are asymptomatic at the detection, and its diagnosis remains extremely difficult when no structured follow-up is offered (5, 27).

Considering the high overall recurrence rate and the insidious clinical presentation, multidisciplinary management and follow-up of CDH patients are recommended, and it is advisable to consider specific follow-up algorithms depending on the patient's risk of recurrence (11). However, it is unclear if active searching with periodic imaging is warranted in all patients for timely recognition of the complication since unnecessary radiation could be avoided in those with low recurrence risk (16).

Since recurrence could occur at any time during the years following primary repair, it would be helpful to promote a remote follow-up that includes a multidisciplinary team of neonatologists, pediatrics, and pediatric surgeons at 3, 6, 12, 18, 24 months of life and then annually until the age of 8 years (12, 47). In addition, standardization of clinical and radiological assessments should be implemented, even for asymptomatic patients (5, 47). CXR should be scheduled at 12 and 24 months and performed anytime as needed, based on the patient's clinical symptoms. Then, it should be planned every 2 years until 8 years old for primary closure, with an additional 18-month CXR for patients undergoing patch repair (47). The preferred diagnostic exams to detect a CDH recurrence are the upper gastrointestinal (UGI) contrast study, barium enema, and computed tomography (CT) scan (54).

CONCLUSIONS AND FUTURE PERSPECTIVES

There is a diffuse agreement that a tension-free diaphragmatic repair with the use of a cone/dome-shaped patch is advisable to reduce the risk of hernia recurrence during the patient's growth (29). However, no specific data definitively show the superiority of biological or synthetic patches (32, 53, 55, 56). The PTFE appears to be associated with a low recurrence rate and is recommended by international groups (10, 57). However, it would be helpful to perform randomized control trials to demonstrate its superiority over absorbable patches (7, 31).

Tissue engineering seems to be the final answer to the search for a perfect diaphragmatic replacement, but many issues still need to be addressed to optimize these techniques for clinical practice (36).

A careful imaging evaluation before patients' discharge is necessary, especially when relevant risk factors for recurrence are present, such as MIS or extensive defect repair (2).

Ultimately, the centralization of CDH patients to referral high-volume centers is pivotal to manage possible complications with an appropriate and customized follow-up plan (23).

AUTHOR CONTRIBUTIONS

FM, GC, GR, IA, MI, and LM contributed to the study's conception and design. FM, GR, MI, IA, and GC wrote the first draft of the manuscript. FM and GR contributed equally and had the right to list their names first in their Curriculum Vitae. IA, JE, LM, and LW provided extensive critical revision. All authors contributed to the manuscript's critical revision, read and approved the submitted version.

REFERENCES

1. Macchini F, Morandi A, Mazzoleni S, Ichino M, Cavallaro G, Raffaeli G, et al. Is fetal endoscopic tracheal occlusion (FETO) a predisposing factor for acid gastro-esophageal reflux in infants with congenital diaphragmatic hernia? *Front Pediatr*. (2020) 8:e00467. doi: 10.3389/fped.2020.00467
2. Putnam LR, Gupta V, Tsao K, Davis CF, Lally PA, Lally KP, et al. Factors associated with early recurrence after congenital diaphragmatic hernia repair. *J Pediatr Surg.* (2017) 52:928–32. doi: 10.1016/j.jpedsurg.2017.03.011
3. Rocha G, Azevedo I, Pinto JC, Guimarães H. Follow-up of the survivors of congenital diaphragmatic hernia. *Early Hum Dev.* (2012) 88:255–8. doi: 10.1016/j.earlhumdev.2011.08.025
4. Lally KP, Engle W, American Academy of Pediatrics Section on Surgery, American Academy of Pediatrics Committee on Fetus and Newborn. Postdischarge follow-up of infants with congenital diaphragmatic hernia. *Pediatrics.* (2008) 121:627–32. doi: 10.1542/peds.2007-3282
5. Nagata K, Usui N, Terui K, Takayasu H, Goishi K, Hayakawa M, et al. Risk factors for the recurrence of the congenital diaphragmatic hernia—report from the long-term follow-up study of Japanese CDH study group. *Eur J Pediatr Surg.* (2015) 25:9–14. doi: 10.1055/s-0034-1395486
6. Tsai J, Sulkowski J, Adzick NS, Hedrick HL, Flake AW. Patch repair for congenital diaphragmatic hernia: is it really a problem? *J Pediatr Surg.* (2012) 47:637–41. doi: 10.1016/j.jpedsurg.2011.11.054
7. Jawaid W, Qasem E, Jones M, Shaw N, Losty P. Outcomes following prosthetic patch repair in newborns with congenital diaphragmatic hernia. *Br J Surg.* (2013) 100:1833–7. doi: 10.1002/bjs.9306
8. Snoek KG, Capolupo I, Morini F, van Rosmalen J, Greenough A, van Heijst A, et al. Score for neonatal acute physiology-II predicts outcome in congenital diaphragmatic hernia patients. *Pediatr Crit Care Med.* (2016) 17:540–6. doi: 10.1097/PCC.0000000000000738
9. van den Hout L, Schaible T, Cohen-Overbeek T, Hop W, Siemer J, van de Ven K, et al. Actual outcome in infants with congenital diaphragmatic hernia: the role of a standardized postnatal treatment protocol. *Fetal Diagn Ther.* (2011) 29:55–63. doi: 10.1159/000322694
10. Snoek KG, Reiss IK, Greenough A, Capolupo I, Urlesberger B, Wessel L, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH EURO consortium consensus-2015 update. *Neonatology.* (2016) 110:66–74. doi: 10.1159/000444210
11. IJsselstijn H, Breatnach C, Hoskote A, Greenough A, Patel N, Capolupo I, et al. Defining outcomes following congenital diaphragmatic hernia using standardised clinical assessment and management plan (SCAMP) methodology within the CDH EURO consortium. *Pediatr Res.* (2018) 84:181–9. doi: 10.1038/s41390-018-0063-3
12. Al-Iede MM, Karpelowsky J, Fitzgerald DA. Recurrent diaphragmatic hernia: modifiable and non-modifiable risk factors. *Pediatr Pulmonol.* (2016) 51:394–401. doi: 10.1002/ppul.23305
13. Gerall CD, Stewart LA, Price J, Kabagambe S, Sferra SR, Schmaedick MJ, et al. Long-term outcomes of congenital diaphragmatic hernia: a single institution experience. *J Pediatr Surg.* (2021). doi: 10.1016/j.jpedsurg.2021.06.007. [Epub ahead of print].
14. Stephenson B, Stamatakis J. Late recurrence of a congenital diaphragmatic hernia. Case report. *Br J Obstet Gynaecol.* (1991) 98:110–1. doi: 10.1111/j.1471-0528.1991.tb10324.x
15. Rowe DH, Stolar CJ. Recurrent diaphragmatic hernia. *Semin Pediatr Surg.* (2003) 12:107–9. doi: 10.1016/S1055-8586(02)00020-3
16. Fisher JC, Haley MJ, Ruiz-Elizalde A, Stolar CJ, Arkovitz MS. Multivariate model for predicting recurrence in congenital diaphragmatic hernia. *J Pediatr Surg.* (2009) 44:1173–80. doi: 10.1016/j.jpedsurg.2009.02.043
17. Amodeo I, Pesenti N, Raffaeli G, Macchini F, Condo V, Borzani I, et al. NeoAPACHE II. Relationship between radiographic pulmonary area and pulmonary hypertension, mortality, and hernia recurrence in newborns with CDH. *Front Pediatr.* (2021) 9:692210. doi: 10.3389/fped.2021.692210
18. Kamran A, Zendejas B, Demehri FR, Nath B, Zurakowski D, Smithers CJ. Risk factors for recurrence after thoracoscopic repair of congenital diaphragmatic hernia (CDH). *J Pediatr Surg.* (2018) 53:2087–91. doi: 10.1016/j.jpedsurg.2018.04.007
19. Levesque M, Derraugh G, Schantz D, Morris MI, Shawyer A, Lum Min SA, et al. The presence of a hernia sac in isolated congenital diaphragmatic hernia is associated with less disease severity: a retrospective cohort study. *J Pediatr Surg.* (2019) 54:899–902. doi: 10.1016/j.jpedsurg.2019.01.016
20. Reiss I, Schaible T, van den Hout L, Capolupo I, Allegaert K, van Heijst A, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH EURO Consortium consensus. *Neonatology.* (2010) 98:354–64. doi: 10.1159/000320622
21. Ali K, Dassios T, Khaliq SA, Williams EE, Tamura K, Davenport M, et al. Outcomes of infants with congenital diaphragmatic hernia by side of defect in the FETO era. *Pediatr Surg Int.* (2019) 35:743–7. doi: 10.1007/s00383-019-04484-3
22. Schaible T, Kohl T, Reinshagen K, Brade J, Neff KW, Stressig R, et al. Right- versus left-sided congenital diaphragmatic hernia: postnatal outcome at a specialized tertiary care center. *Pediatr Crit Care Med.* (2012) 13:66–71. doi: 10.1097/PCC.0b013e3182192aa9
23. Cioci AC, Urrechaga EM, Parreco J, Remer LF, Cowan M, Perez EA, et al. One-year outcomes of congenital diaphragmatic hernia repair: factors associated with recurrence and complications. *J Pediatr Surg.* (2021) 56:1542–6. doi: 10.1016/j.jpedsurg.2020.09.018
24. De Bie F, Suply E, Verbelen T, Vanstraelen S, Debeer A, Cross K, et al. Early surgical complications after congenital diaphragmatic hernia repair by thoracotomy vs. laparotomy: A bicentric comparison. *J Pediatr Surg.* (2020) 55:2105–10. doi: 10.1016/j.jpedsurg.2019.12.020
25. Heiwegen K, Van Rooij IALM, Van Heijst A, De Blaauw I, Botden SMBI. Surgical complications in children with CDH: a multivariate analysis. *World J Surg.* (2020) 44:2042–8. doi: 10.1007/s00268-020-05387-4
26. Heiwegen K, De Blaauw I, Botden SMBI. A systematic review and meta-analysis of surgical morbidity of primary versus patch repaired congenital diaphragmatic hernia patients. *Sci Rep.* (2021) 11:12661. doi: 10.1038/s41598-021-91908-7
27. Zahn KB, Schaible T, Rafat N, Weis M, Weiss C, Wessel L. Longitudinal follow-up with radiologic screening for recurrence and secondary hiatal hernia in neonates with open repair of congenital diaphragmatic hernia—a large prospective, observational cohort study at one referral center. *Front Pediatr.* (2021) 9:e796478. doi: 10.3389/fped.2021.796478
28. Zani A, Zani-Ruttenstock E, Pierro A. Advances in the surgical approach to congenital diaphragmatic hernia. *Semin Fetal Neonatal Med.* (2014) 19:364–9. doi: 10.1016/j.siny.2014.09.002
29. Suply E, Rees C, Cross K, Elagami H, Blackburn S, Giuliani S, et al. Patch repair of congenital diaphragmatic hernia is not at risk of poor outcomes. *J Pediatr Surg.* (2020) 55:1522–7. doi: 10.1016/j.jpedsurg.2019.10.021
30. Vandewalle RJ, Yalcin S, Clifton MS, Wulkan ML. Biologic mesh underlay in thoracoscopic primary repair of congenital diaphragmatic hernia confers reduced recurrence in neonates: a preliminary report. *J Laparoendosc Adv Surg Techn.* (2019) 29:1212–5. doi: 10.1089/lap.2019.0122
31. Camila GR, Aixa R, Carlos G, Mariano B, Soledad A, Gisela S, et al. Biological versus synthetic patch for the repair of congenital diaphragmatic hernia: 8-year experience at a tertiary center. *J Pediatr Surg.* (2021) 56:1957–61. doi: 10.1016/j.jpedsurg.2021.02.052
32. Nasr A, Struijs M-C, Ein SH, Langer JC, Chiu PPL. Outcomes after muscle flap vs. prosthetic patch repair for large congenital diaphragmatic hernias. *J Pediatr Surg.* (2010) 45:151–4. doi: 10.1016/j.jpedsurg.2009.10.024
33. Barnhart DC, Jacques E, Scaife ER, Yoder BA, Meyers RL, Harman A, et al. Split abdominal wall muscle flap repair vs. patch repair of large congenital diaphragmatic hernias. *J Pediatr Surg.* (2012) 47:81–6. doi: 10.1016/j.jpedsurg.2011.10.023
34. Trevisan C, Maghin E, Dedja A, Caccin P, de Cesare N, Franzin C, et al. Allogenic tissue-specific decellularized scaffolds promote long-term muscle innervation and functional recovery in a surgical diaphragmatic hernia model. *Acta Biomater.* (2019) 89:115–25. doi: 10.1016/j.actbio.2019.03.007
35. Fauza DO, editor. Tissue engineering in congenital diaphragmatic hernia. *Semin Pediatr Surg.* (2014) 23:135–40. doi: 10.1053/j.sempedsurg.2014.04.004
36. Saxena AK. Surgical perspectives regarding application of biomaterials for the management of large congenital diaphragmatic hernia defects. *Pediatr Surg Int.* (2018) 34:475–89. doi: 10.1007/s00383-018-4253-1
37. Grethel EJ, Cortes RA, Wagner AJ, Clifton MS, Lee H, Farmer DL, et al. Prosthetic patches for congenital diaphragmatic hernia repair: surgisis vs. Gore-Tex. *J Pediatr Surg.* (2006) 41:29–33. doi: 10.1016/j.jpedsurg.2005.10.005

38. Short HL, Clifton MS, Arps K, Travers C, Loewen J, Schlager A. The "Flat Diaphragm": does the degree of curvature of the diaphragm on postoperative X-Ray predict congenital diaphragmatic hernia recurrence? *J Laparoendosc Adv Surg Techn.* (2018) 28:476–80. doi: 10.1089/lap.2017.0228
39. Okawada M, Ohfuji S, Yamoto M, Urushihara N, Terui K, Nagata K, et al. Thoracoscopic repair of congenital diaphragmatic hernia in neonates: findings of a multicenter study in Japan. *Surg Today*. (2021) 51:1694–702. doi: 10.1007/s00595-021-02278-6
40. Cho SD, Krishnaswami S, McKee JC, Zallen G, Silen ML, Bliss DW. Analysis of 29 consecutive thoracoscopic repairs of congenital diaphragmatic hernia in neonates compared to historical controls. *J Pediatr Surg.* (2009) 44:80–6. doi: 10.1016/j.jpedsurg.2008.10.013
41. Gander JW, Fisher JC, Gross ER, Reichstein AR, Cowles RA, Aspelund G, et al. Early recurrence of congenital diaphragmatic hernia is higher after thoracoscopic than open repair: a single institutional study. *J Pediatr Surg.* (2011) 46:1303–8. doi: 10.1016/j.jpedsurg.2010.11.048
42. Gourlay DM, Cassidy LD, Sato TT, Lal DR, Arca MJ. Beyond feasibility: a comparison of newborns undergoing thoracoscopic and open repair of congenital diaphragmatic hernias. *J Pediatr Surg.* (2009) 44:1702–7. doi: 10.1016/j.jpedsurg.2008.11.030
43. Lacher M, St Peter SD, Laje P, Harmon CM, Ure B, Kuebler JF. Thoracoscopic CDH repair—a survey on opinion and experience among IPEG members. *J Laparoendosc Adv Surg Techn.* (2015) 25:954–7. doi: 10.1089/lap.2015.0243
44. Okazaki T, Nishimura K, Takahashi T, Shoji H, Shimizu T, Tanaka T, et al. Indications for thoracoscopic repair of congenital diaphragmatic hernia in neonates. *Pediatr Surg Int.* (2011) 27:35–8. doi: 10.1007/s00383-010-2724-0
45. Szavay PO, Obermayr F, Maas C, Luenig H, Blumenstock G, Fuchs J. Perioperative outcome of patients with congenital diaphragmatic hernia undergoing open versus minimally invasive surgery. *J Laparoendosc Adv Surg Tech.* (2012) 22:285–9. doi: 10.1089/lap.2011.0356
46. Wagner R, Mayer S, Feng X, Gosemann J-H, Zimmermann P, Lacher M. Thoracoscopic repair of congenital diaphragmatic hernia. *Eur J Pediatr Surg.* (2020) 30:137–41. doi: 10.1055/s-0040-1702222
47. De Haro Jorge I, Prat Ortells J, Martín-Solé O, Muñoz Fernandez E, Pertierra A, Martin-Lluis A, et al. Porcine dermal patches as a risk factor for recurrence after congenital diaphragmatic hernia repair. *Pediatr Surg Int.* (2021) 37:59–65. doi: 10.1007/s00383-020-04787-w
48. Shieh HF, Wilson JM, Sheils CA, Smithers CJ, Kharasch VS, Becker RE, et al. Does the ex utero intrapartum treatment to extracorporeal membrane oxygenation procedure change morbidity outcomes for high-risk congenital diaphragmatic hernia survivors? *J Pediatr Surg.* (2017) 52:22–5. doi: 10.1016/j.jpedsurg.2016.10.010
49. Abramov A, Fan W, Hernan R, Zenilman AL, Wynn J, Aspelund G, et al. Comparative outcomes of right versus left congenital diaphragmatic hernia: a multicenter analysis. *J Pediatr Surg.* (2020) 55:33–8. doi: 10.1016/j.jpedsurg.2019.09.046
50. Bucher BT, Guth RM, Saito JM, Najaf T, Warner BW. Impact of hospital volume on in-hospital mortality of infants undergoing repair of congenital diaphragmatic hernia. *Ann Surg.* (2010) 252:635–42. doi: 10.1097/SLA.0b013e3181f5b538
51. Bruns NE, Glenn IC, McNinch NL, Arps K, Ponsky TA, Schlager A. Approach to recurrent congenital diaphragmatic hernia: results of an international survey. *J Laparoendosc Adv Surg Tech.* (2016) 26:925–9. doi: 10.1089/lap.2016.0247
52. Moss RL, Chen CM, Harrison MR. Prosthetic patch durability in congenital diaphragmatic hernia: a long-term follow-up study. *J Pediatr Surg.* (2001) 36:152–4. doi: 10.1053/jpsu.2001.20037
53. Laituri C, Garey C, Valusek P, Fike F, Kaye A, Ostlie D, et al. Outcome of congenital diaphragmatic hernia repair depending on patch type. *Eur J Pediatr Surg.* (2010) 20:363–5. doi: 10.1055/s-0030-1261939
54. Dewberry L, Hilton S, Gien J, Liechty KW, Marwan AI. Flap repair in congenital diaphragmatic hernia leads to lower rates of recurrence. *J Pediatr Surg.* (2019) 54:2487–91. doi: 10.1016/j.jpedsurg.2019.08.042
55. Romao RLP, Nasr A, Chiu PPL, Langer JC. What is the best prosthetic material for patch repair of congenital diaphragmatic hernia? Comparison and meta-analysis of porcine small intestinal submucosa and polytetrafluoroethylene. *J Pediatr Surg.* (2012) 47:1496–500. doi: 10.1016/j.jpedsurg.2012.01.009
56. Jancelewicz T, Vu LT, Keller RL, Bratton B, Lee H, Farmer D, et al. Long-term surgical outcomes in congenital diaphragmatic hernia: observations from a single institution. *J Pediatr Surg.* (2010) 45:155–60. doi: 10.1016/j.jpedsurg.2009.10.028
57. Puligandla PS, Grabowski J, Austin M, Hedrick H, Renaud E, Arnold M, et al. Management of congenital diaphragmatic hernia: a systematic review from the APSA outcomes and evidence based practice committee. *J Pediatr Surg.* (2015) 50:1958–70. doi: 10.1016/j.jpedsurg.2015.09.010

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REVIEW
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Challenges and Pitfalls: Performing Clinical Trials in Patients With Congenital Diaphragmatic Hernia

Suzan Cochius - den Otter[1]*, *Jan A. Deprest*[2,3,4], *Laurent Storme*[5,6,7], *Anne Greenough*[8,9,10,11] *and Dick Tibboel*[1]

[1] Intensive Care and Department of Pediatric Surgery, Erasmus MC University Medical Center, Rotterdam, Netherlands, [2] Department of Obstetrics and Gynaecology, University Hospitals KU Leuven, Leuven, Belgium, [3] Academic Department of Development and Regeneration, Biomedical Sciences, KU Leuven, Leuven, Belgium, [4] Institute for Women's Health, University College London, London, United Kingdom, [5] Metrics-Perinatal Environment and Health, University of Lille, Lille, France, [6] Department of Neonatology, Jeanne de Flandre Hospital, Centre Hospitalier Universitaire de Lille, Lille, France, [7] Center of Rare Disease Congenital Diaphragmatic Hernia, Jeanne de Flandre Hospital, Centre Hospitalier Universitaire de Lille, Lille, France, [8] Neonatal Intensive Care Centre, King's College Hospital NHS Foundation Trust, London, United Kingdom, [9] Department of Women and Children's Health, School of Life Course Sciences, Faculty of Life Sciences and Medicine, King's College London, London, United Kingdom, [10] The Asthma UK Centre for Allergic Mechanisms in Asthma, King's College London, London, United Kingdom, [11] NIHR Biomedical Centre, Guy's and St Thomas NHS Foundation Trust and King's College London, London, United Kingdom

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***Correspondence:**
Suzan Cochius - den Otter
s.denotter@erasmusmc.nl

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Congenital diaphragmatic hernia (CDH) is a rare developmental defect of the lungs and diaphragm, with substantial morbidity and mortality. Although internationally established treatment guidelines have been developed, most recommendations are still expert opinions. Trials in patients with CDH, more in particular randomized controlled trials, are rare. Only three multicenter trials in patients with CDH have been completed, which focused on fetoscopic tracheal occlusion and ventilation mode. Another four are currently recruiting, two with a focus on perinatal transition and two on the treatment of pulmonary hypertension. Herein, we discuss major challenges and pitfalls when performing a clinical trial in infants with CDH. It is essential to select the correct intervention and dose, select the appropriate population of CDH patients, and also define a relevant endpoint that allows a realistic duration and sample size. New statistical approaches might increase the feasibility of randomized controlled trials in patients with CDH. One should also timely perform the trial when there is still equipoise. But above all, awareness of policymakers for the relevance of investigator-initiated trials is essential for future clinical research in this rare disease.

Keywords: congenital diaphragmatic hernia, clinical trials, congenital anomaly, prenatal therapy, postnatal therapy

INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a rare developmental defect of the lungs and diaphragm that occurs in 1 per 4,000–4,500 live births (1). Infants with CDH are born with a variable amount of lung hypoplasia and abnormal pulmonary vasculature, causing ventilatory insufficiency and pulmonary hypertension (PH). Nowadays, with the introduction of standardized care, survival is ∼73% in well-established centers of expertise (2). Although these centers use internationally established treatment guidelines, most recommendations are still expert opinions (3, 4). Trials in patients with CDH, more in particular randomized

controlled trials (RCTs), are rare (2, 5–10). Sometimes, patients with CDH are included in RCTs, with a subanalysis for the patients with CDH, although the trial may not be powered to be informative for the latter (11, 12). More often, CDH is an exclusion criterion for participation in an RCT (13, 14). When trials are successfully completed, extrapolation of the results to clinical practice becomes a matter of debate (15). This way, relevant research questions stay unanswered, or their conclusions remain questioned and hence are not implemented.

TRIALS IN CONGENITAL DIAPHRAGMATIC HERNIA

Over time, the focus of therapy and thus the research questions in CDH have changed. Until the 1980s, CDH was considered a surgical emergency. Thereafter, preoperative stabilization became mainstream, focusing on correcting acidosis and hypoxia (16). The use of aggressive ventilation strategies, however, in the hypoplastic lung caused barotrauma and a high incidence of pneumothorax. Wung et al. (17) reported a respiratory strategy to minimize the risk of iatrogenic lung injury and exacerbation of PH, which focused on the prevention of hyperventilation and hyperinflation. Since then, this strategy has been adopted worldwide, and surgical closure has changed into an elective procedure in "stable" patients. In 2010, the first postnatal management guidelines of the CDH EURO Consortium were published, initiating standardized care throughout Europe (18). Together with the introduction of these guidelines, the VICI trial started the first postnatal RCT exclusively in CDH patients. In this CDH Euro Consortium trial, conventional ventilation and high-frequency oscillatory ventilation were compared (19). The primary outcome was chronic lung disease and/or mortality on day 28. Unfortunately, the calculated number of 400 patients was never included. The study was concluded after the recruitment of 171 patients over a study period of 5 years, because of lower-than-anticipated inclusion rate, logistic issues with recruitment in different centers, lack of financial resources, and increasing fear for trial fatigue. The study showed no significant difference between ventilation modes but was underpowered to make this a firm conclusion. Other outcome parameters, including treatment failure, ventilation time, duration of inotropic support, and need for extracorporeal membrane oxygenation (ECMO), all showed a trend toward a more favorable outcome in the conventional ventilation group 2 (2). In parallel, prenatal interventions were developed, based on the assumption that the diagnosis can be made prenatally, and the severity can be assessed. These were initially based on the anatomical repair by open fetal surgery but later focused on the stimulation of lung development [reviewed in (20)]. The latter can be achieved by temporary tracheal occlusion, *via* a percutaneous approach. The first, single-center, RCT assessing improvement in survival following tracheal occlusion using a variety of techniques in fetuses with severe and moderate hypoplasia was finalized early because of a higher-than-expected survival in the postnatal management group and a high prematurity rate in the intervention group (10). The European centers that designed the percutaneous fetoscopic occlusion technique with a balloon [fetoscopic endoluminal tracheal occlusion (FETO)] (21), which is maternally more acceptable, moved from a phase I trial to a large cohort study (22, 23). In view of the apparently higher survival rates compared to historical controls (24) but the lack of evidence, they initiated the Tracheal Occlusion To Accelerate Lung growth (TOTAL) randomized clinical trials. The trials were performed in fetuses with severe and moderate hypoplasia born in expert fetoscopy centers that also used the standardized neonatal management protocol as defined by the CDH Euro consortium (3). In severe left-sided CDH, FETO performed between 27 and 29 weeks significantly improved survival at discharge from the neonatal intensive care unit [relative risk (RR): 2.67 (95% CI: 1.22–6.11)], however, with increased risk of prematurity [RR: 2.59 (1.59–4.52)] (5). In patients with moderate CDH, FETO performed between 30 and 32 weeks did not improve survival [RR: 1.27 (0.99–1.63)] or need for oxygen at 6 months of age, at increased risk of prematurity [RR: 2.86 (1.94–4.34)] (6). In a pooled analysis of the data, the overall survival following FETO is increased [RR: 1.78 (1.05–3.01)], and it seems that the difference between both trials may be due to the difference in the time point of balloon insertion (25). In retrospect, there was preclinical and some observational clinical evidence that earlier occlusion yields a more vigorous lung response, but at the same time, it increases prematurity risk; hence, it was debated as being a good strategy (26, 27). The risk of tracheomalacia secondary to tracheal occlusion was low in both trials, with an incidence of 1.9% (5, 6, 28).

While the hypoplastic lung is still a relevant problem, nowadays, the focus has shifted somewhat from the lung toward PH, which remains an important determinant of mortality and morbidity (29). There are currently two trials recruiting that focus on physiological-based cord clamping: the PinC trial (NCT04373902) and the CHIC trial (9). Neonatal resuscitation of infants with CDH remains highly challenging because of the failure of cardiorespiratory adaptation at birth. The baby is frequently cyanotic and bradycardic as soon as the umbilical cord is clamped. Traditionally, the umbilical cord is clamped and cut immediately after birth. Following cord clamping, umbilical venous return is lost, and left ventricular output becomes dependent on pulmonary blood flow. However, in CDH infants, an increase in pulmonary blood flow is delayed because of high pulmonary vascular resistance. Delaying cord clamping while the resuscitation maneuvers are started may (1) facilitate blood transfer from the placenta to the baby to augment circulatory blood volume; (2) avoid the loss of venous return and decrease in left ventricle filling caused by immediate cord clamping; and (3) prevent initial hypoxemia because of sustained uteroplacental gas exchange after birth when the cord is intact. The PINC trial, performed in Europe, focuses on decreasing the incidence of PH, defined as 2 out of the 4 following criteria: (1) right ventricular systolic pressure (RVSP) $\geq$2/3 systemic systolic pressure, (2) right ventricle (RV) dilatation/septal displacement or RV dysfunction +/– left ventricle dysfunction, (3) pre–post ductal SpO_2 difference >10%, and (4) oxygenation index (OI) >20. In this trial, a standardized echocardiogram is implemented. In the CHIC trial conducted in the French Rare

Disease Network, the aim is to investigate the efficacy of intact cord resuscitation on cardiorespiratory adaptation directly after birth by comparing APGAR score (9).

In parallel, two RCTs are recruiting CDH neonates in the search for the best initial therapy for PH (7, 8). In the CoDiNOS trial (7), again initiated within the CDH Euro Consortium, intravenous sildenafil is compared with iNO as initial therapy for PH in CDH patients. In this trial, PH is strictly defined, using the same criteria as in the PinC trial. Structural and standardized echocardiograms are performed at set times, with the additional aim of increasing the knowledge of PH and cardiac function in CDH patients. In the Milrinone in CDH trial, a trial performed within the Neonatal Research Network in the United States, milrinone is compared with placebo in CDH patients with mild-to-moderate PH, defined as an OI of 10 or higher (**Table 1**) (8).

CHALLENGES WHEN PERFORMING TRIALS IN PATIENTS WITH CONGENITAL DIAPHRAGMATIC HERNIA

The Right Intervention at the Right Time for the Right Patient

When performing a trial in patients with CDH, either prenatal or postnatal using a pharmacological intervention, it is essential to first establish an adequate dosing regimen before evaluating efficacy. Although pharmacokinetic drug testing in adults is very common, in infants, dosing regimens are often an extrapolation from adult data, only corrected for body size (30). This assumes that fractioning of the dose will lead to similar plasma drug levels, hence assuming that children have similar renal, gastrointestinal, and hepatic functions as well as body composition as adults. This can result in over- or under-dosing, consequently leading to toxicity or reduced efficacy (31). For sildenafil, a pharmacokinetic model in infants with CDH was built, using a NONMEM approach, before starting a trial (32). With a loading dose of 0.4 mg/kg in 3 h followed by a continuous infusion of 1.6 mg/kg/day, adequate sildenafil plasma levels were achieved, 190 μg/L after the loading dose. The numbers, however, were too low to detect any correlation between these concentrations and the OI. Earlier, Steinhorn et al. tested this dosing regimen in a dose-escalation trial in infants with persistent PH of the newborn (PPHN), defined as signs of PH on echocardiography, an OI > 15, and no other anomalies (33). Again, numbers were too low to detect a strong correlation between the different dosing regimens, plasma concentrations, and clinical effects. But patients with a plasma concentration over 58 μg/L after the loading dose seemed to have a decreased OI 4 h later. Recently, Pierce et al. reported in the same population, newborns with PPHN and no other anomalies, no significant additional effect of sildenafil to iNO in the treatment of PPHN in an RCT (34). The dosing regimen was the second-lowest regimen that improved the OI in the study by Steinhorn et al. (33). Improvement, however, was observed in a combined set of, mostly higher, dosing regimens. Steady-state concentrations of this combined group were 123 ng/ml, but only 73 μg/ml in the group using this lower sildenafil dosing regimen. In the recent trial of Pierce et al. the steady-state concentration was only 52 μg/ml (34). One can assume that the plasma concentration should at least be 123 ng/ml in order to observe clinical effects, underlining the necessity of pharmacokinetic modeling. So the question remains whether sildenafil has an additional effect in patients with PPHN who are already treated with iNO and whether sildenafil was dosed appropriately in the trial by Pierce et al. Samples collected during the CoDiNOS trial will provide more insight into the dose–response correlation of sildenafil in CDH patients as well as its other pharmacodynamic effects.

But is sildenafil the right drug? Although sildenafil could play a role in the treatment of PH in CDH, one could argue that, from a pathophysiological standpoint, it would be more logical

TABLE 1 | Randomized controlled trials in CDH.

RCT	Started in	Intervention	Primary outcome
Fetal tracheal occlusion (10)*	1999–2001	FETO vs. standard prenatal care for moderate to severe CDH	Survival at age of 90 days
VICI trial (2)	2008–2013	Conventional ventilation vs. high-frequency ventilation	Death until discharge or CLD on day 28
TOTAL trial (6)	2008–2019	FETO vs. standard prenatal care for moderate CDH	Infant survival until discharge from intensive care and survival without oxygen at 6 months
TOTAL trial (5)	2011–2020	FETO vs. standard prenatal care for severe CDH	Infant survival until discharge from intensive care
Milrinone in CDH (8)	2016	Milrinone vs. placebo	Change in OI after 24 h
CDH Optimisation of Neonatal Ventilation*	2016	Ventilation with different tidal volumes	Change in pressure time product of the diaphragm
CoDiNOS (7)	2017	Sildenafil vs. iNO	Change in OI after 12 h
PinC	2020	Physiological-based cord clamping vs. direct cord clamping	Incidence of PH in the first day of life
CHIC (9)	2020	Physiological-based cord clamping vs. immediate cord clamping	Apgar score at 1 and 5 min
HFO vs. HFJ ventilation*	2021	High-frequency oscillatory ventilation vs. high-frequency jet ventilation	OI at 24 h

CDH, congenital diaphragmatic hernia; CLD, chronic lung disease; FETO, fetoscopic endoluminal tracheal occlusion; PH, pulmonary hypertension; iNO, inhaled nitric oxide; OI, oxygenation index; HFO, high-frequency oscillation; HFJ, high-frequency jet ventilation; RCT, randomized controlled trial.
**Single-center trial.*

to investigate drugs that act on different pathways, instead of comparing drugs that act on the nitric oxide–cGMP pathway such as sildenafil and iNO. Furthermore, no alteration of the nitric oxide–cGMP pathway in CDH patients has been found, decreasing the chance that drugs acting on this pathway will be effective (35). In a clinical retrospective trial, sildenafil seems beneficial in less than half of the patients with CDH (36). Drugs that affect the endothelin pathway, such as bosentan, might be more successful. An increase in endothelin A and B receptor expression and ECE-1 enzyme is found in patients with CDH. This enzyme is responsible for the conversion of endothelin-1 to its active form (35). CDH patients with PH have higher endothelin-1 plasma levels than CDH patients without PH (37). But endothelin receptor antagonists are still only available in oral form, making them unsuitable for the treatment of postnatal PH in CDH patients before surgical correction. The third pathway involved in PH, the prostacyclin pathway, seems to be altered in CDH patients too, with a decrease of prostaglandin-I2 receptor expression. This could explain the negative effect of prostacyclin derivates on PH in CDH patients, although results are conflicting (35, 38, 39). To decrease the incidence of PH, sildenafil has been discredited for its use prenatally, even though preclinical data in animals with CDH are promising. The Dutch STRIDER study, a trial investigating the effect of sildenafil on fetal growth restriction unrelated to CDH, was suspended because of an increased incidence of PPHN and neonatal mortality (40). It is, however, questionable if these negative findings should be extrapolated to other conditions. Antenatal administered sildenafil reduces vascular branching in healthy fetal rabbits but decreases the incidence of PH in animals with CDH by increasing the number of peripheral vessels (41). A phase I–IIb was set up to evaluate *in vivo* transplacental passage of sildenafil in humans and specifically in infants with CDH (42–44). But despite the preclinical differences, this trial had to be halted unduly after the publication of the results of the STRIDER trial.

PH in CDH is often resistant to pulmonary vasodilators such as iNO. This is possibly due to coexisting right and left ventricular dysfunction (36, 45). Milrinone has both inotropic and lusitropic properties and potentially decreases pulmonary vascular resistance (46). In the trial currently recruiting, infants with CDH and an OI of >10 are randomized for milrinone or placebo. The primary outcome is a change in OI over 24 h. In 2011 and 2012, only 17% of infants born in centers within the NRN, a well-established US research network, received milrinone (8). But currently, it is often common practice, and this could decrease the willingness within the NRN to participate in the trial, decreasing recruitment rates.

The CoDiNOS trial is also suffering from recruiting issues, and this is partly caused by lower-than-anticipated recruitment due to strict inclusion criteria. Although an echocardiogram is often believed to be the best diagnostic tool in newborns, the incidence of PH on echocardiogram on day 1 of life overestimates the incidence of clinically relevant PH. High pulmonary pressures at that time are still part of the physiological transition. Only infants with clinically relevant PH, defined as PH on echocardiogram and clinical signs of PH, are included. This definition decreases the eligible population from 60% to around 30%. Although this negatively affects the inclusion rate, including mild cases dilutes the effect of an intervention. Moreover, the harm of intervention for these mild cases should be taken into account, although the side effects of sildenafil seem to be mild (47). The same problem applies to prenatal and perinatal interventions. Even though ultrasound and MRI have made it possible to identify the severity of lung hypoplasia in infants with CDH, it is still very difficult to predict the severity of PH, due to the difference in pre- and postnatal physiology (48). Better prenatal diagnostic techniques should be able to identify the fetuses and newborns at risk and predict who would benefit from entry in a clinical trial. This would improve the safety and efficacy of a trial. A core outcome set with strictly defined and relevant outcome parameters is currently under development for perinatal interventions (49). A core outcome set for postnatal interventions and long-term outcomes would help to be able to compare postnatal trials and their outcome.

Is Congenital Diaphragmatic Hernia, a Heterogeneous Orphan Disease, a Condition That Is Amenable for a Trial Anyway?

But is an RCT as we know it in its present form, in heterogeneous orphan diseases such as CDH, the only or optimal tool to collect evidence-based information (50–53)? The VICI trial had recruitment problems and lacked adequate financial support. Also, the TOTAL trials were not financially supported apart from the setup of the database. This seriously affected the research infrastructure in participating centers. Currently, lack of financial support has a serious impact on the CoDiNOS trial. Other important limiting factors for recruitment are the delays caused by national drug authorities' approval in participating countries and problems with legislation and insurance. Many centers were so far unable to join the CoDiNOS trial, although the primarily responsible physicians did see the relevance of participating in such a trial. Performing an RCT in pediatric and neonatal critical care is challenging, especially when a high number of centers are needed due to the rarity of the condition or eligible study participants. Collaborating in a research network, such as the CDH EURO Consortium, increases the chance of success. Members of the consortium are often collaborating as one team with a common goal, helping each other to overcome local and national barriers (54). The regulatory framework conduct (Randomized) Clinical Trials in pregnant women and children, especially drug interventions or new medical devices, are increasingly stringent and differ between countries. For example, many countries and healthcare institutions insist researchers use a Clinical Trial Organization or to perform external safety audits, but these organizations and audits are often very expensive, absorbing a major part of the already limited budget of investigator-initiated trials. Interestingly enough, investigator-initiated research is significantly more frequently cited than industry-led trials (55). This demonstrates that investigator-initiated research is essential and has an impact on clinical practice, as data are generated in a real-world setting. Legislation should adjust to facilitate such trials instead of being obstructive,

often without any proven benefit or added safety. This was acknowledged in the revision of the Directive of the European Commission in 2014, "getting better legislation in place soon is crucial to enable and encourage life-saving research," but this did not result in a substantial practice change (56). In January 2022, the new European Clinical Trial Regulation will be implemented, to simplify and accelerate clinical trials within the European Union. By centrally submitting the study protocols for the European Union and synchronizing the leap time for the different medical ethics review processes, study centers in different countries can start recruiting subjects at the same time (57). With this regulation, conducting trials will hopefully become less complex within Europe. But in the TOTAL trial, centers from outside the European Union participated, and also in the CoDiNOS trial, centers from the United Kingdom, Australia, and Canada unsuccessfully intended to join. Worldwide research networks using a uniform approach concerning protocols and outcome measures as well as getting the regulatory bodies to cooperate and agree on uniformity in research procedures would improve the research climate for rare diseases such as CDH tremendously.

As to CDH, which is a rare disease, one would hope that the European Reference Networks, launched in 2017, would facilitate clinical research, which was amongst others one of the goals of ERNs. One of these networks is the European Reference Network for rare Inherited and Congenital (digestive and gastrointestinal) Anomalies (ERNICA). The CDH EURO Consortium, which has been existing longer and has a proven track record, is now affiliated with ERNICA, which may help to increase funding and resources. ERNs include patient organizations, but the latter did not wait and have been and still are involved in the funding and development of investigator-initiated trials. Their participation increases the clinical relevance of trials. For instance, the CoDiNOS trial is funded for an important part by CDH-UK, the CDH patient organization in the United Kingdom.

Another factor is the heterogeneous severity of the condition (from very mild to very severe). A large number of patients as well as stratification based on prenatal markers of severity are required to show statistical differences. For instance, for the CHIC trial, an estimated 600 infants are needed to demonstrate a difference in mortality. That is unrealistic, and thus proxies are being used as the primary outcome. It is likely that physiological-based cord clamping will become standard of care if the PINC or CHIC shows a statistical difference in primary endpoints, even without evidence of a decrease in mortality rate. The same applies to the CoDiNOS trial, in which the initial primary endpoint (incidence of PH at the age of 2 weeks) was changed to change in OI at 12 h. That lowered the patients needed from 330 to 90, without decreasing the relevance of the trial. In many neonatal trials on PPHN, OI is used as primary outcome (13, 14). Not only the severity of the condition is heterogeneous, but also the outcome between centers differs, and centers that treat a low number of patients have a worse outcome than the high-volume centers with both complex neonatal intensive care facilities and expertise in neonatal surgery (58). A benefit of performing trials within, for instance, the CDH Euro Consortium, is that the affiliated centers are expert high-volume centers that offer standardized care, improving the baseline outcome of patients, although differences in outcome still exist (59).

Potentially statistical approaches can increase the feasibility of trials in rare diseases. In an early phase, $n = 1$ trials can be used to explore causality. Platform trials, often with a long duration, are commonly used in oncology. The major advantage is the ability to evaluate multiple interventions and the possibility to drop treatment arms and add new ones (60). But also new statistical techniques are being developed for controlled trials. For instance, one could decrease the number of included patients needed to achieve statistical significance by adding real-world controls to a trial. These real-world controls consist of patients who were not included in the trial due to logistic and organizational issues or whose parents chose not to participate in the trial. Considering the VICI trial, more than 425 of the 619 CDH patients who were treated in the VICI trial centers were not included in the trial. These real-world controls would be highly comparable to the VICI trial patients, as they share the same treatment period and the same treatment guideline (i.e., the CDH EURO consortium guidelines), and they were treated in the same centers. Most of the patients not participating were initially treated with conventional mechanical ventilation, because this was standard of care at the time. When combining data from the VICI trial with the observational data from the real-world controls, one needs to account for potential differences in baseline patient characteristics and other biases that may arise from the inclusion of nonrandomized data. In the TOTAL trials, for instance, the outcome in the nonparticipating patients differs from that of the participating patients (5, 6). For these issues, different statistical techniques such as dynamic borrowing can be used (61). This approach would lead to revised estimates of the treatment effect of ventilation mode on the primary endpoint with greater statistical power and precision. Using real-world controls could substantially increase the feasibility of RCTs in a rare patient population. To our knowledge, however, it has not been used in clinical research.

One may also need fewer patients by using a more sensitive primary endpoint than a dichotomous or cross-sectional endpoint. One could incorporate repeated measurements or have a more informative scale (e.g., ordinal or continuous), as it increases the statistical power. For instance, CLD was defined in the VICI trial as the need for any respiratory support on day 28. This definition disregards the amount and the duration of respiratory support. Several additional measurements collected from VICI trial patients could be used to define more informative endpoints. This can include continuous variables such as the degree of oxygen support required, ordinal endpoints such as the level of ventilation support, and derived endpoints such as time to discharge or time to the reduction of ventilatory support. Based on these informative endpoints, multiple hypothesis tests with improved statistical properties compared to the original primary analysis can be applied. One can test each endpoint separately but also combine the endpoints in a single composite endpoint, for instance, by defining a score that incorporates information from the different endpoints and accounts for mortality. Specific statistical approaches to account for multiplicity for testing of multiple, repeatedly measured endpoints will be needed, for

instance, the multiple marginal generalized estimating equation (GEE) model method (62). This method can incorporate endpoints on different scales (e.g., death and oxygen support), record measurements at a single time point only, and perform repeated measurements while taking the correlation between endpoints into account to maximize the power of statistical tests. Consultation with biostatisticians at an early stage of trial design is increasingly important to prevent frustration and loss of contributing centers by conducting a classical RCT, especially as newer statistical approaches are on the horizon. The equations should be inserted in editable format from the equation editor.

CONCLUSION

So far, only three multicenter clinical trials have been shown to be clinically possible, i.e., two prenatal and one postnatal trials, all with a ventilatory endpoint (2, 5, 6). These trials were very difficult to conduct. New RCTs are recruiting, and those focus on the reduction in PH, a major contributor to mortality in CDH patients. Our experience learns that performing an RCT in CDH patients is challenging. One should timely perform the trial when there is still equipoise. It is essential to select the correct intervention and dose, select the appropriate population of CDH patients, and define a relevant endpoint that also allows a realistic duration and sample size. Also, new statistical approaches might increase the feasibility of RCTs in patients with CDH. But above all, awareness of policymakers for the relevance of investigator-initiated trials should be increased. Possibly European Reference Networks, such as ERNICA, can have a role in improving the climate for these trials. After the implementation of the new European Clinical Trial Regulation, regulators should timely evaluate the effects on investigator-initiated trials, especially in rare diseases such as CDH.

AUTHOR CONTRIBUTIONS

SC-dO drafted the initial manuscript and reviewed and revised the manuscript. JD, LS, AG, and DT critically revised the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. McGivern MR, Best KE, Rankin J, Wellesley D, Greenlees R, Addor MC, et al. Epidemiology of congenital diaphragmatic hernia in Europe: a register-based study. *Arch Dis Child Fetal Neonatal Ed.* (2015) 100:F137–44. doi: 10.1136/archdischild-2014-306174
2. Snoek KG, Capolupo I, van Rosmalen J, Hout Lde J, Vijfhuize S, Greenough A, et al. Conventional mechanical ventilation versus high-frequency oscillatory ventilation for congenital diaphragmatic hernia: a randomized clinical trial (The VICI-trial). *Ann Surg.* (2016) 263:867–74. doi: 10.1097/SLA.0000000000001533
3. Snoek KG, Reiss IK, Greenough A, Capolupo I, Urlesberger B, Wessel L, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: The CDH EURO Consortium Consensus-−2015 update. *Neonatology.* (2016) 110:66–74. doi: 10.1159/000444210
4. LaRusso K, Baird R, Keijzer R, Skarsgard E, Puligandla P. Standardizing congenital diaphragmatic hernia care in Canada: implementing national clinical practice guidelines. *J Pediatr Surg.* (2020) 55:835–43. doi: 10.1016/j.jpedsurg.2020.01.021
5. Deprest JA, Nicolaides KH, Benachi A, Gratacos E, Ryan G, Persico N, et al. Randomized trial of fetal surgery for severe left diaphragmatic hernia. *N Engl J Med.* (2021) 385:107–18. doi: 10.1056/NEJMoa2027030
6. Deprest JA, Benachi A, Gratacos E, Nicolaides KH, Berg C, Persico N, et al. Randomized trial of fetal surgery for moderate left diaphragmatic hernia. *N Engl J Med.* (2021) 385:119–29. doi: 10.1056/NEJMoa2026983
7. Cochius-den Otter S, Schaible T, Greenough A, van Heijst A, Patel N, Allegaert K, et al. The CoDiNOS trial protocol: an international randomised controlled trial of intravenous sildenafil versus inhaled nitric oxide for the treatment of pulmonary hypertension in neonates with congenital diaphragmatic hernia. *BMJ Open.* (2019) 9:e032122. doi: 10.1136/bmjopen-2019-032122
8. Lakshminrusimha S, Keszler M, Kirpalani H, Van Meurs K, Chess P, Ambalavanan N, et al. Milrinone in congenital diaphragmatic hernia – a randomized pilot trial: study protocol, review of literature and survey of current practices. *Matern Health Neonatol Perinatol.* (2017) 3:27. doi: 10.1186/s40748-017-0066-9
9. Le Duc K, Mur S, Rakza T, Boukhris MR, Rousset C, Vaast P, et al. Efficacy of intact cord resuscitation compared to immediate cord clamping on cardiorespiratory adaptation at birth in infants with isolated congenital diaphragmatic hernia (CHIC). *Children.* (2021) 8:e339. doi: 10.3390/children8050339
10. Harrison MR, Keller RL, Hawgood SB, Kitterman JA, Sandberg PL, Farmer DL, et al. A randomized trial of fetal endoscopic tracheal occlusion for severe fetal congenital diaphragmatic hernia. *N Engl J Med.* (2003) 349:1916–24. doi: 10.1056/NEJMoa035005
11. The Neonatal Inhaled Nitric Oxide Study Group (NINOS). Inhaled nitric oxide and hypoxic respiratory failure in infants with congenital diaphragmatic hernia. *Pediatrics.* (1997) 99:838–45. doi: 10.1542/peds.99.6.838
12. UK Collaborative ECMO Trail Group. UK collaborative randomised trial of neonatal extracorporeal membrane oxygenation. *Lancet.* (1996) 348:75–82. doi: 10.1016/S0140-6736(96)04100-1
13. Kelly LE, Ohlsson A, Shah PS. Sildenafil for pulmonary hypertension in neonates. *Cochrane Database Syst Rev.* (2017) 8:CD005494. doi: 10.1002/14651858.CD005494.pub4
14. Barrington KJ, Finer N, Pennaforte T, Altit G. Nitric oxide for respiratory failure in infants born at or near term. *Cochrane Database Syst Rev.* (2017) 1:CD000399. doi: 10.1002/14651858.CD000399.pub3
15. Stolar CJH, Flake AW, Losty PD. Fetal surgery for severe left diaphragmatic hernia. *N Engl J Med.* (2021) 385:2111–2. doi: 10.1056/NEJMc2115673
16. Cartlidge PH, Mann NP, Kapila L. Preoperative stabilisation in congenital diaphragmatic hernia. *Arch Dis Child.* (1986) 61:1226–8. doi: 10.1136/adc.61.12.1226
17. Wung JT, Sahni R, Moffitt ST, Lipsitz E, Stolar CJ. Congenital diaphragmatic hernia: survival treated with very delayed surgery, spontaneous respiration, and no chest tube. *J Pediatr Surg.* (1995) 30:406–9. doi: 10.1016/0022-3468(95)90042-X
18. Reiss I, Schaible T, van den Hout L, Capolupo I, Allegaert K, van Heijst A, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH EURO Consortium consensus. *Neonatology.* (2010) 98:354–64. doi: 10.1159/000320622
19. van den Hout L, Tibboel D, Vijfhuize S. te Beest H, Hop W, Reiss I, et al. The VICI-trial: high frequency oscillation versus conventional mechanical ventilation in newborns with congenital diaphragmatic hernia: an international multicentre randomized controlled trial. *BMC Pediatr.* (2011) 11:98. doi: 10.1186/1471-2431-11-98

20. Deprest JA, Flake AW, Gratacos E, Ville Y, Hecher K, Nicolaides K, et al. The making of fetal surgery. *Prenat Diagn.* (2010) 30:653–67. doi: 10.1002/pd.2571
21. Flageole H, Evrard VA, Vandenberghe K, Lerut TE, Deprest JA. Tracheoscopic endotracheal occlusion in the ovine model: technique and pulmonary effects. *J Pediatr Surg.* (1997) 32:1328–31. doi: 10.1016/S0022-3468(97)90314-6
22. Jani JC, Nicolaides KH, Gratacos E, Valencia CM, Done E, Martinez JM, et al. Severe diaphragmatic hernia treated by fetal endoscopic tracheal occlusion. *Ultrasound Obstet Gynecol.* (2009) 34:304–10. doi: 10.1002/uog.6450
23. Deprest J, Gratacos E, Nicolaides KH, Group FT. Fetoscopic tracheal occlusion (FETO) for severe congenital diaphragmatic hernia: evolution of a technique and preliminary results. *Ultrasound Obstet Gynecol.* (2004) 24:121–6. doi: 10.1002/uog.1711
24. Jani J, Nicolaides KH, Keller RL, Benachi A, Peralta CF, Favre R, et al. Observed to expected lung area to head circumference ratio in the prediction of survival in fetuses with isolated diaphragmatic hernia. *Ultrasound Obstet Gynecol.* (2007) 30:67–71. doi: 10.1002/uog.4052
25. Van Calster B, Benachi A, Nicolaides KH, Gratacos E, Berg C, Persico N, et al. The randomized TOTAL-trials on fetal surgery for congenital diaphragmatic hernia: re-analysis using pooled data. *Am J Obstet Gynecol.* (2021) 226:560e1–24. doi: 10.1016/j.ajog.2021.11.1351. [Epub ahead of print].
26. Kohl T, Gembruch U, Filsinger B, Hering R, Bruhn J, Tchatcheva K, et al. Encouraging early clinical experience with deliberately delayed temporary fetoscopic tracheal occlusion for the prenatal treatment of life-threatening right and left congenital diaphragmatic hernias. *Fetal Diagn Ther.* (2006) 21:314–8. doi: 10.1159/000091363
27. Nawapun K, Eastwood MP, Diaz-Cobos D, Jimenez J, Aertsen M, Gomez O, et al. In vivo evidence by magnetic resonance volumetry of a gestational age dependent response to tracheal occlusion for congenital diaphragmatic hernia. *Prenat Diagn.* (2015) 35:1048–56. doi: 10.1002/pd.4642
28. Russo FM, Cordier AG, Basurto D, Salazar L, Litwinska E, Gomez O, et al. Fetoscopic endoluminal tracheal occlusion reverses the natural history of right-sided congenital diaphragmatic hernia: a European multicenter experience. *Ultrasound Obstet Gynecol.* (2021) 57:378–85. doi: 10.1002/uog.23115
29. Pierro M, Thebaud B. Understanding and treating pulmonary hypertension in congenital diaphragmatic hernia. *Semin Fetal Neonatal Med.* (2014) 19:357–63. doi: 10.1016/j.siny.2014.09.008
30. Vermeulen E, van den Anker JN, Della Pasqua O, Hoppu K, van der Lee JH, Global Research in P. How to optimise drug study design: pharmacokinetics and pharmacodynamics studies introduced to paediatricians. *J Pharm Pharmacol.* (2017) 69:439–47. doi: 10.1111/jphp.12637
31. Benjamin DK, Smith PB, Jadhav P, Gobburu JV, Murphy MD, Hasselblad V, et al. Pediatric antihypertensive trial failures—analysis of end points and dose range. *Hypertension.* (2008) 51:834–40. doi: 10.1161/HYPERTENSIONAHA.107.108886
32. Cochius-den Otter SCM, Kipfmueller F, de Winter BCM, Allegaert K, Tibboel D, Mueller A, et al. Pharmacokinetic modeling of intravenous sildenafil in newborns with congenital diaphragmatic hernia. *Eur J Clin Pharmacol.* (2019) 76:219–27. doi: 10.1007/s00228-019-02767-1
33. Steinhorn RH, Kinsella JP, Pierce C, Butrous G, Dilleen M, Oakes M, et al. Intravenous sildenafil in the treatment of neonates with persistent pulmonary hypertension. *J Pediatr.* (2009) 155:841–7 e1. doi: 10.1016/j.jpeds.2009.06.012
34. Pierce CM, Zhang MH, Jonsson B, Iorga D, Cheruvu N, Balagtas CC, et al. Efficacy and safety of IV sildenafil in the treatment of newborn infants with, or at risk of persistent pulmonary hypertension of the newborn (PPHN): a multicenter, randomized, placebo-controlled trial. *J Pediatr.* (2021) 237:154–61e3. doi: 10.1016/j.jpeds.2021.05.051
35. Mous DS. Buscop-van Kempen MJ, Wijnen RMH, Tibboel D, Rottier RJ. Changes in vasoactive pathways in congenital diaphragmatic hernia associated pulmonary hypertension explain unresponsiveness to pharmacotherapy. *Respir Res.* (2017) 18:187. doi: 10.1186/s12931-017-0670-2
36. Kipfmueller F, Schroeder L, Berg C, Heindel K, Bartmann P, Mueller A. Continuous intravenous sildenafil as an early treatment in neonates with congenital diaphragmatic hernia. *Pediatr Pulmonol.* (2018) 53:452–60. doi: 10.1002/ppul.23935
37. Keller RL, Tacy TA, Hendricks-Munoz K, Xu J, Moon-Grady AJ, Neuhaus J, et al. Congenital diaphragmatic hernia: endothelin-1, pulmonary hypertension, and disease severity. *Am J Respir Crit Care Med.* (2010) 182:555–61. doi: 10.1164/rccm.200907-1126OC
38. Skarda DE, Yoder BA, Anstadt EE, Lally PA, Greene T, McFadden M, et al. Epoprostenol does not affect mortality in neonates with congenital diaphragmatic hernia. *Eur J Pediatr Surg.* (2015) 25:454–9. doi: 10.1055/s-0034-1389096
39. Lawrence KM, Hedrick HL, Monk HM, Herkert L, Waqar LN, Hanna BD, et al. Treprostinil improves persistent pulmonary hypertension associated with congenital diaphragmatic hernia. *J Pediatr.* (2018) 200:44–9. doi: 10.1016/j.jpeds.2018.04.052
40. Groom KM, Ganzevoort W, Alfirevic Z, Lim K, Papageorghiou AT, Consortium S. Clinicians should stop prescribing sildenafil for fetal growth restriction (FGR): comment from the STRIDER Consortium. *Ultrasound Obstet Gynecol.* (2018) 52:295–6. doi: 10.1002/uog.19186
41. Kashyap AJ, DeKoninck PLJ, Rodgers KA, Thio M, McGillick EV, Amberg BJ, et al. Antenatal sildenafil treatment improves neonatal pulmonary hemodynamics and gas exchange in lambs with diaphragmatic hernia. *Ultrasound Obstet Gynecol.* (2019) 54:506–16. doi: 10.1002/uog.20415
42. Russo FM, Hooper S, Tibboel D, DeKoninck P, Benachi A, Treluyer JM, et al. Antenatal therapy with sildenafil: don't throw the baby out with the bathwater. *Ultrasound Obstet Gynecol.* (2019) 53:274–5. doi: 10.1002/uog.20201
43. Russo FM, De Bie F, Hodges R, Flake A, Deprest J. Sildenafil for antenatal treatment of congenital diaphragmatic hernia: from bench to bedside. *Curr Pharm Des.* (2019) 25:601–8. doi: 10.2174/1381612825666190320151856
44. Russo FM, Benachi A, Van Mieghem T, De Hoon J, Van Calsteren K, Annaert P, et al. Antenatal sildenafil administration to prevent pulmonary hypertension in congenital diaphragmatic hernia (SToP-PH): study protocol for a phase I/IIb placenta transfer and safety study. *Trials.* (2018) 19:524. doi: 10.1186/s13063-018-2897-8
45. Lawrence KM, Monos S, Adams S, Herkert L, Peranteau WH, Munson DA, et al. Inhaled nitric oxide is associated with improved oxygenation in a subpopulation of infants with congenital diaphragmatic hernia and pulmonary hypertension. *J Pediatr.* (2020) 219:167–72. doi: 10.1016/j.jpeds.2019.09.052
46. Lakshminrusimha S, Steinhorn RH. Inodilators in nitric oxide resistant persistent pulmonary hypertension of the newborn. *Pediatr Crit Care Med.* (2013) 14:107–9. doi: 10.1097/PCC.0b013e318250af44
47. Samiee-Zafarghandy S, Smith PB, van den Anker JN. Safety of sildenafil in infants*. *Pediatr Crit Care Med.* (2014) 15:362–8. doi: 10.1097/PCC.0000000000000077
48. Russo FM, Eastwood MP, Keijzer R, Al-Maary J, Toelen J, Van Mieghem T, et al. Lung size and liver herniation predict need for extracorporeal membrane oxygenation but not pulmonary hypertension in isolated congenital diaphragmatic hernia: systematic review and meta-analysis. *Ultrasound Obstet Gynecol.* (2017) 49:704–13. doi: 10.1002/uog.16000
49. Vergote S, De Bie F, Bosteels J, Hedrick H, Duffy J, Power B, et al. Study protocol: a core outcome set for perinatal interventions for congenital diaphragmatic hernia. *Trials.* (2021) 22:158. doi: 10.1186/s13063-021-05120-z
50. Ville Y. Should we offer fetal surgery for severe congenital diaphragmatic hernia or bring these cases to trial? Difference between chance and hazard. *Ultrasound Obstet Gynecol.* (2020) 56:491–2. doi: 10.1002/uog.22103
51. Rodrigues HC, Deprest J, Berg PP. When referring physicians and researchers disagree on equipoise: the TOTAL trial experience. *Prenat Diagn.* (2011) 31:589–94. doi: 10.1002/pd.2756
52. Deprest J. Prenatal treatment of severe congenital diaphragmatic hernia: there is still medical equipoise. *Ultrasound Obstet Gynecol.* (2020) 56:493–7. doi: 10.1002/uog.22182
53. Vergote S, Pizzolato D, Russo F, Dierickx K, Deprest J, Crombag N. The TOTAL trial dilemma: a survey among professionals on equipoise regarding fetal therapy for severe congenital diaphragmatic hernia. *Prenat Diagn.* (2021) 41:179–89. doi: 10.1002/pd.5849
54. Duffett M, Choong K, Foster J, Meade M, Menon K, Parker M, et al. High-quality randomized controlled trials in pediatric critical care: a

survey of barriers and facilitators. *Pediatr Crit Care Med.* (2017) 18:405–13. doi: 10.1097/PCC.0000000000001144
55. Marshall JC, Kwong W, Kommaraju K, Burns KE. Determinants of citation impact in large clinical trials in critical care: the role of investigator-led clinical trials groups. *Crit Care Med.* (2016) 44:663–70. doi: 10.1097/CCM.0000000000001466
56. European Union. *Clinical Trials: Clearer Rules, Better Protection* [press release] (2015).
57. EMA. *EMA European Clinical Trial Regulation 2022*. (2021). Available online at: https://www.ema.europa.eu/en/human-regulatory/research-development/clinical-trials/clinical-trials-regulation#:~:text=The%20Regulation%20harmonises%20the%20assessment,Trials%20Information%20System%20(CTIS).&text=When%20the%20Regulation%20becomes%20applicable,Trials%20Directive%20(EC)%20No (accessed March 14, 2022).
58. Apfeld JC, Kastenberg ZJ, Gibbons AT, Carmichael SL, Lee HC, Sylvester KG. Treating center volume and congenital diaphragmatic hernia outcomes in California. *J Pediatr.* (2020) 222:146–53.e1. doi: 10.1016/j.jpeds.2020.03.028
59. Snoek KG, Greenough A, van Rosmalen J, Capolupo I, Schaible T, Ali K, et al. Congenital diaphragmatic hernia: 10-year evaluation of survival, extracorporeal membrane oxygenation, and foetoscopic endotracheal occlusion in four high-volume centres. *Neonatology.* (2018) 113:63–8. doi: 10.1159/000480451
60. Park JJH, Harari O, Dron L, Lester RT, Thorlund K, Mills EJ. An overview of platform trials with a checklist for clinical readers. *J Clin Epidemiol.* (2020) 125:1–8. doi: 10.1016/j.jclinepi.2020.04.025
61. Viele K, Berry S, Neuenschwander B, Amzal B, Chen F, Enas N, et al. Use of historical control data for assessing treatment effects in clinical trials. *Pharm Stat.* (2014) 13:41–54. doi: 10.1002/pst.1589
62. Ristl R, Hothorn L, Ritz C, Posch M. Simultaneous inference for multiple marginal generalized estimating equation models. *Stat Methods Med Res.* (2020) 29:1746–62. doi: 10.1177/0962280219873005

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Small Bowel Obstruction After Neonatal Repair of Congenital Diaphragmatic Hernia—Incidence and Risk-Factors Identified in a Large Longitudinal Cohort-Study

Katrin B. Zahn[1,2*†], *Anna-Maria Franz*[1,2†], *Thomas Schaible*[3], *Neysan Rafat*[3], *Sylvia Büttner*[4], *Michael Boettcher*[1,2] *and Lucas M. Wessel*[1,2]

[1] Department of Pediatric Surgery, University Children's Hospital Mannheim, University of Heidelberg, Mannheim, Germany, [2] ERNICA Centre, Mannheim, Germany, [3] Department of Neonatology, University Children's Hospital Mannheim, University of Heidelberg, Mannheim, Germany, [4] Department of Medical Statistics and Biomathematics, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany

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***Correspondence:**
Katrin B. Zahn
katrin.zahn@umm.de

[†] These authors share first authorship

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Objective: In patients with a congenital diaphragmatic hernia (CDH), postoperative small bowel obstruction (SBO) is a life-threatening event. Literature reports an incidence of SBO of 20% and an association with patch repair and ECMO treatment. Adhesions develop due to peritoneal damage and underly various biochemical and cellular processes. This longitudinal cohort study is aimed at identifying the incidence of SBO and the risk factors of surgical, pre-, and postoperative treatment.

Methods: We evaluated all consecutive CDH survivors born between January 2009 and December 2017 participating in our prospective long-term follow-up program with a standardized protocol.

Results: A total of 337 patients were included, with a median follow-up of 4 years. SBO with various underlying causes was observed in 38 patients (11.3%) and significantly more often after open surgery (OS). The majority of SBOs required surgical intervention (92%). Adhesive SBO (ASBO) was detected as the leading cause in 17 of 28 patients, in whom surgical reports were available. Duration of chest tube insertion [odds ratio (OR) 1.22; 95% CI 1.01–1.46, $p = 0.04$] was identified as an independent predictor for ASBO in multivariate analysis. Beyond the cut-off value of 16 days, the incidence of serous effusion and chylothorax was higher in patients with ASBO (ASBO/non-SBO: 2/10 vs. 3/139 serous effusion, $p = 0.04$; 2/10 vs. 13/139 chylothorax, $p = 0.27$). Type of diaphragmatic reconstruction, abdominal wall closure, or ECMO treatment showed no significant association with ASBO. A protective effect of one or more re-operations has been detected (RR 0.16; 95% CI 0.02–1.17; $p = 0.049$).

Conclusion: Thoracoscopic CDH repair significantly lowers the risk of SBO; however, not every patient is suitable for this approach. GoreTex®-patches do not seem to affect the development of ASBO, while median laparotomy might be more favorable than a subcostal incision. Neonates produce more proinflammatory cytokines and have

a reduced anti-inflammatory capacity, which may contribute to the higher incidence of ASBO in patients with a longer duration of chest tube insertion, serous effusion, chylothorax, and to the protective effect of re-operations. In the future, novel therapeutic strategies based on a better understanding of the biochemical and cellular processes involved in the pathophysiology of adhesion formation might contribute to a reduction of peritoneal adhesions and their associated morbidity and mortality.

Keywords: longitudinal follow-up, intestinal complications, congenital diaphragmatic hernia (CDH), adhesions, adhesive small bowel obstruction (SBO), risk factors

INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a rare malformation of an incompletely formed diaphragm. Depending on the size of the defect and its association with major cardiac anomalies, survival rates vary from 99 to 39% (1). It is assumed that due to advances in treatment and with the application of standardized treatment protocols, the overall survival improved, even in severely diseased infants (2, 3). Therefore, CDH-associated morbidity due to pulmonary hypoplasia, pulmonary hypertension, gastroesophageal reflux, musculoskeletal abnormalities, and impaired neurodevelopment have drawn more attention (2, 4).

Among these long-term sequelae, adhesive small bowel obstruction (ASBO) occurs as a life-threatening event after surgical reconstruction of the diaphragm, but objective data are scarce. In general, the type of surgery and the extent of peritoneal damage are considered the most important risk factors for SBO due to adhesions (5). Also, other triggers for adhesion-related readmissions including peritonitis, previous surgery, or patient age have been described (6). Taken together, reduced fibrinolysis, increased fibrin formation, procoagulatory status, and enhanced inflammation seem to be the most important factors in the pathophysiology of adhesion formation in general (7). In neonates, there are additional factors contributing to the formation of adhesions: a reduced production of anti-inflammatory cytokines and a diminished response to anti-inflammatory stimuli in preterm and post-term infants have been reported (8). Additionally, in neonates with CDH complicated by pulmonary hypertension, increased levels of adhesion molecules that play an important role in the inflammatory and immunologic response were detected (9). Therefore, immaturity of the immune system in the neonatal period and pulmonary hypertension inherent to CDH may support the formation of abdominal adhesions in neonates with CDH due to an imbalance of the humoral and cellular system with proinflammatory tendency.

In adults, postoperative adhesions were found in 93% of patients, who had one or more previous abdominal operations (10). Adhesions can be defined as strands or membranes of fibrous tissue that connect various intra-abdominal organs, which are normally separated (5, 11). However, adhesions can be asymptomatic or cause symptoms, such as abdominal pain, altered bowel habits, bloating, or intestinal obstruction, which may be either partial or complete (12, 13). The ASBO seems to be associated with a substantial risk of morbidity (circulatory disturbances, gangrenous bowel, perforation, need for bowel resection, and septicaemia) (14–18) and mortality in children (14), which is nowadays mainly attributed to overwhelming sepsis or other comorbidities (16).

For children, the reported incidence of postoperative bowel obstruction requiring further laparotomy varies from 3.3 to 8.3% in patients, who had previously undergone laparotomy in the neonatal period (19, 20). In infants undergoing Ladd's procedure for malrotation, which was associated with CDH, duodenal atresia, gastroschisis, or esophageal atresia, ASBO even occurred in 14.9% (20). However, there is a large variation in the incidence of ASBO depending on the procedure, localization within the abdominal cavity, and patient age (21). A higher incidence of up to 4.7% was shown in children younger than 1 year compared to 2.1% for older children (22). The risk of developing ASBO seems even higher in neonates (3.3%), compared to infants (1.9%) or older children (1.7%), but with no statistical significance (19). However, different studies agree that most of the adhesive obstructions developed within 1 year of the previous procedure but were also observed later (20–22).

Regarding patients with CDH, Yokota et al. reported that neonates who underwent subcostal laparotomy for the reconstruction of the diaphragm required re-operation for intestinal adhesion obstruction significantly more often than patients who underwent other neonatal laparotomies (17.6% vs. 6.7%, $p = 0.02$) (23). A previously performed retrospective study on long-term surgical morbidity in CDH survivors presented an incidence of about 20% for SBO, with a mean follow-up of 7.3 years (24).

Especially in CDH, there are other causes for SBO besides adhesive formations, like duodenal kinking, Ladd's bands, volvulus, or incarceration due to recurrence. At least 45% of patients with CDH have an associated intestinal rotation abnormality (25). This abnormal rotation of the embryological midgut leads to a nonfixation of the right colon, resulting in aberrant attempts of fixation (Ladd's bands) and could cause intestinal passage disruption with the clinical presentation of SBO (25, 26). Also, the sole attachment of the intestine predisposes for volvulus (26). Due to these anatomical characteristics inherent to CDH, patients show a higher risk for volvulus. In 0.3% of CDH survivors, a volvulus occurred within 1.5 years after the reconstruction of the diaphragm (27).

This longitudinal cohort study aimed at identifying the incidence of SBO and ASBO as well as risk factors of surgical,

TABLE 1 | Schedule of our standardized follow-up program.

	Birth	½ y	1 y	2 y	4 y	6 y	10 y	14 y	18 y
Chest X-ray	X	X	X	-	X	X	-	X	-
ECG	X	X	X	X	X	X	X	X	X
Cardiac ECHO	X	X	X	X	X	X	X	X	X
MRI	-	-	-	ECMO	-	-	X	-	-
Lowdose CT	-	-	-	Non ECMO	-	-	-	-	X
Pulmonary function	-	-	-	-	-	X	X	X	X
Ophthalmology	X	-	X	-	-	-	-	-	-
Hearing test	X	-	X	-	-	-	-	-	-
Neurodevelopmental assessment	-	X	X	X	X	X	-	-	-

y, year; ECG, electrocardiogram; ECHO, echocardiography; MRI, magnetic resonance imaging; CT, computed tomography; ECMO, extracorporeal membrane oxygenation.

pre-, and postoperative treatment in children with neonatal repair of CDH.

MATERIALS AND METHODS

Study Group

Consecutive neonates with CDH born from 1 January 2009 to 31 December 2017 and treated at our neonatal intensive care unit (NICU) at the Department of Neonatology of the University Children's Hospital Mannheim, University of Heidelberg, were included in this prospective follow-up study. This study was approved by our local ethics committee (2018-592N-MA) and informed consent was obtained from parents. Our standardized long-term follow-up program has been designed to observe the development of CDH survivors from their childhood until adolescence (**Table 1**).

Treatment of all infants born with CDH was based on the guidelines of the CDH-Euro-Consortium (28, 29). Surgical repair of all patients has been performed after hemodynamic stabilization. The approach (midline laparotomy vs. minimal-invasive) was chosen depending on the estimated size of the defect and cardiopulmonary stability of the patient. In patients undergoing laparotomy, a cone-shaped GoreTex®-patch was used for larger defects to create a tension-free closure of the diaphragm (30). Also, if the primary closure of the laparotomy would be too tight, a GoreTex®-patch was implanted into the abdominal wall to prevent abdominal compartment syndrome. Also, for patients with a minimal-invasive reconstruction of the diaphragm, GoreTex®-patches were used in cases with a missing lateral diaphragmatic rim.

Patients, who underwent diaphragmatic reconstruction at another institution or received surgical treatment after 28 days of life, were excluded. Since SBO has been reported to develop mainly within 1 year after the previous surgery, another exclusion criterion was the follow-up of <1 year in patients without SBO. Data were collected until October 2019 and analyzed for SBO and possible risk factors of demographics, surgical, and pre- and post-operative treatment (ethics vote 2019-1151R).

Adhesive SBO has been defined as a partial or complete intestinal obstruction depending on the symptomatology and eligibility for conservative treatment to achieve relief of symptoms and re-establishment of enteral nutrition. Conservative treatment comprised abstinence from oral food, placement of a nasogastric tube, repeated enemas, and parenteral rehydration under close clinical re-evaluation. In cases with signs of impaired circulation or suspected perforation, deterioration of symptoms and/or missing improvement under conservative treatment over more than 3 days or in patients with suspected volvulus or CDH-recurrence, the indication for re-operation was made.

Statistical Analysis

All data were entered into a Microsoft Excel database and patients were pseudonymized by numbers. Quantitative values were presented by median, minimum, and maximum as well as qualitative values by number (n) and percentage (%). Therefore, the study cohort was separated into patients with and patients without SBO and ASBO. Differences in the results of these groups were assessed for statistical significance using χ^2- and Fisher's exact test for qualitative data, or rather U- and t-test for quantitative data. A $p < 0.05$ was considered statistically significant. Odds ratio (OR), as a measure of the effect of the characteristics on SBO, and the likelihood were calculated for quantitative data using logistic regression analysis in case of a significant result. For qualitative data, the relative risk (RR) for the occurrence of SBO was described. In addition, we performed a multivariate analysis to demonstrate the independence of possible risk factors. The analysis was performed using SAS v14.2 [Statistical Analysis System, Version 14.2 (SAS Institute Inc., Cary, North Carolina, USA)] with grateful support from the Department of Medical Statistics and Biomathematics, Medical Faculty Mannheim.

RESULTS

Study Cohort

A consort diagram of our study cohort is presented in **Figure 1**. A total of 516 patients were identified, of which 84 (16.3%) deceased before surgical repair and 12 (2.3%) were late presenting. Therefore, 420 patients were eligible for this study, of whom 26 deceased within the first year of life. Besides 38 patients who developed SBO, 299 patients without SBO completed at

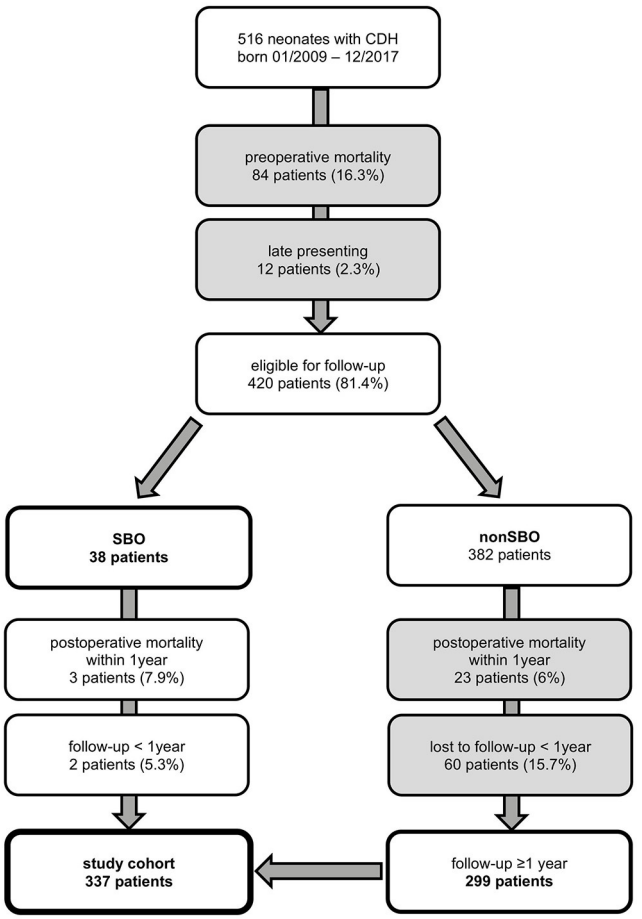

FIGURE 1 | Neonates with congenital diaphragmatic hernia (CDH) born from January 2009 to December 2017 and treated at our institution and participation at follow-up until October 2019 with excluded patients in gray boxes [SBO = small bowel obstruction].

least 1 year of follow-up. Thus, 337 neonates were included for further analysis.

Almost every infant received an antibiotic treatment postnatally (96.1%, $n = 323$). The use of extracorporeal membrane oxygenation (ECMO) was necessary in 139 cases (41.3%) with a median duration of 9.0 days.

Surgical Treatment and Intraoperative Findings

For detailed information on surgical treatment and intraoperative findings, refer to **Table 2**. Minimally invasive surgery (MIS) was successfully completed in 20.5% of patients, of whom the majority received a primary thoracoscopy (98.6%). A conventional open approach with a midline laparotomy was performed in 79.5%, of which 21 patients were converted from an initial minimal invasive approach. There was a predominance of left-sided CDH (84.3%), CDH without a hernial sac (86.7%), and posterior localization of the diaphragmatic defect (Bochdalek's

TABLE 2 | Surgical characteristics and intraoperative findings of the study cohort.

	Study cohort ***n* = 337**
Timing of reconstruction in days – median (min.-max.)	6 (0 - 21)[a]
Surgical time in minutes – median (min.-max.)	174 (58 - 388)[b]
Operation at neonatal intensive care unit	202 (59.9)
Surgical approach – n (%)	
Minimally invasive	69 (20.5)
Open	268 (79.5)
Side of defect – n (%)	
Left side	284 (84.3)
Right side	51 (15.1)
Bilateral	2 (0.6)
Liver-up in left sided CDH– n (%)	160 (56.3)
Hernia type – n (%)	
Discontinuity/without hernia sac	292 (86.7)
With hernia sac	45 (13.4)
Defect size[c] – n (%)	
A	31 (9.2)
B	115 (34.1)
C	158 (46.9)
D	33 (9.8)
Anatomic localisation of the defect – n (%)	
Bochdalek	311 (92.8)[d]
Morgagni or Larrey	24 (7.2)
Reconstruction of the diaphragm – n (%)	
Primary closure	65 (19.3)
Patch correction	272 (80.7)
Abdominal wall closure with patch – n (%)	68 (20.2)
Intraoperative adhesion prevention – n (%)	
Seprafilm®	7 (2.1)
Fibrin	2 (0.6)
Contamination class[e] – n (%)	
1	314 (93.2)
2	22 (6.5)
3	1 (0.3)
Cases with additional operative procedures – n (%)[f]	108 (32.1)[e]
- Release of duodenal kinking	38 (11.3)
- Resection of Meckel's diverticulum	18 (5.3)
- Resection of accessory spleen	18 (5.3)
- Primary fundopexy	16 (4.8)
- Resection of Ladd's bands	13 (3.9)
- Resection of lung sequestration	13 (3.9)
- Resection of accessory liver tissue	12 (3.6)
- Miscellaneous additional procedures[g]	10 (3.0)
- Insertion of stoma	2 (0.6)

CDH, congenital diaphragmatic hernia.
[a] 1 data missing.
[b] 3 data missing.
[c] classified by Lally et al. (1).
[d] 2 data missing.
[e] classified by the CDC: Surgical Wound Classification Grades I–IV (31).
[f] cases received more than one additional procedure.
[g] suture of intestinal perforation, resection of intestine, mesentery adaption, closure of tracheoesophageal fistula, lymph node resection, and suture of pericardial defect.

foramen, 92.8%). Defect size was intraoperatively classified according to the CDH study group (1) in all neonates and mainly large defects were detected in our cohort (C and D: 56.7%). Accordingly, surgical repair of the diaphragm was performed with a GoreTex®-patch in 80.7% of all patients, whereas primary closure was achieved in 19.3%. The difference regarding the type of CDH repair between patients with open surgery (OS) and MIS was significant (patch: 93.7% OS vs. 30.4% MIS; $p < 0.0001$). To prevent abdominal compartment syndrome, a GoreTex®-patch was implanted in the abdominal wall in 25.4% with midline laparotomy. Intraoperative adhesion prevention was used in 3.4% in OS. Mostly, the initial surgery was performed without contamination (93.2%) as classified by the Centers for Disease Control and Prevention (CDC) (31). A total of 32.1% of patients received additional procedures during the surgical reconstruction of the diaphragm.

Re-operations During Follow-Up

During follow-up, 91 patients (27.0%) underwent secondary surgical procedures in the abdominal or thoracic cavity other than for SBO. In total, 62 CDH survivors (18.4%) had one, 19 (5.6%) had two, and eight (2.4%) had three re-operations. In two complicated cases, one patient received seven and one child received eight re-operations over the years. The median time to the first re-operation was 156 days (range 1-1,972), 402 days (range 39–3,193) to the second, and 691.5 days (185–1,942) to the third. Re-operation due to recurrence occurred in 10.4% ($n =$ 35). During follow-up, the implanted abdominal wall patch was excised in 56 of 68 cases (82.4%) and reduced in size in 7 patients (10.3%). For treatment of gastroesophageal reflux, hiatoplasty and fundoplication were performed in 23 cases (6.8%), and other intestinal procedures like resection of the intestine or Meckel's diverticulum, insertion of a jejunal feeding tube or stoma, or pyloromyotomy in 28 cases (8.3%) were performed. A total of 16 patients (17.6%) underwent miscellaneous procedures (reconstruction of umbilical or incisional abdominal wall hernias, resection of tumorous formations, funicolysis of intraabdominal testes, cholecystostomy, laparostomy formation, and partial lung resection due to CPAM or implantation of ventriculoperitoneal shunts). Intraoperative adhesion prevention barriers were used in 17.6% of patients.

Small Bowel Obstruction

During the observation period from January 2009 to October 2019, SBO was observed in a total of 38 patients (11.3%), with a median time to the presentation of 178 days (range, 23–1018). Most were diagnosed in the first year of life ($n = 27$; 71.1%), another seven (18.4%) within the second, and four (10.5%) within the third year after the initial intervention (**Figure 2**). A total of 10 of 38 children showed a partial obstruction (26.3%), of which three could be treated conservatively (30%). There was one child with recurrent SBO, but with incomplete obstruction and conservative treatment, respectively. In total, 26 children were treated at our institution, and the remaining 12 were treated at an outside hospital. The majority of children presenting with SBO needed surgical treatment ($n = 35$; 92.1%). In seven cases (20%), surgical reports did not reveal a distinct cause, or data are missing due to surgery being performed at an outside hospital. Among those with available surgical reports, adhesive bands were identified as a leading cause ($n = 17$; 60.7%). Furthermore, one or more of the following underlying conditions for the symptoms of SBO could be detected: volvulus ($n = 5$; 17.9%), intestinal kinking ($n = 5$, 17.9%), incarceration due to CDH recurrence ($n = 3$; 10.7%), inner herniation ($n = 3$; 10.7%), and Ladd's bands ($n =$ 1; 3.6%).

In 13 patients (46.4%), one or more additional procedures were performed: segmental resection of the intestine ($n = 7$; 25%), antireflux surgery ($n = 4$; 14.3%), jejunal feeding tube placement ($n = 4$; 14.3%), insertion of a stoma ($n = 2$; 7.1%),

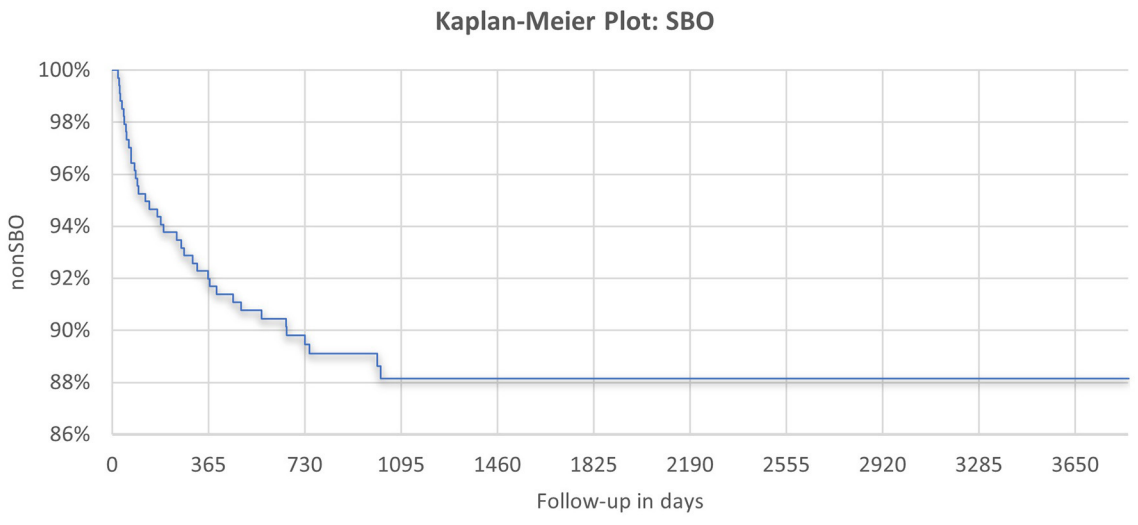

FIGURE 2 | Kaplan–Meier curve for the occurrence of SBO during follow-up with a maximum of 10 years.

or laparostomy formation ($n = 1$; 3.6%). Barriers for adhesion prevention were used five times at re-laparotomy (17.9%).

Median follow-up was 3.7 years (range, 0.4–6.7) for the SBO group, 4.2 years (range, 0.4–6.3) for the ASBO group, and 4.1 years (range, 1.0–10.6) for the non-SBO group. We found no significant differences in demographics, patient characteristics, or preoperative treatment between patients with and without SBO.

Mortality and Morbidity

There was no known mortality associated with the occurrence of SBO or ASBO in our cohort. Bowel resection was necessary in three of 17 patients with ASBO (17.6%).

Surgical Characteristics, Intraoperative Findings, and Postoperative Treatment in SBO

Details of surgical characteristics and intraoperative findings are displayed in **Table 3**. The only significant difference between patients with SBO and non-SBO was detected for the surgical approach: a higher incidence of SBO was observed after open surgery (OS 13.1% vs. MIS 4.4%; $p = 0.04$). The type of diaphragmatic reconstruction (primary vs. patch-repair) showed no difference, neither in the total cohort nor in patients after median laparotomy (4/17 (23.5%) primary vs. 31 of 251 (12.4%) patch-repair, $p = 0.25$). The difference in SBO between patients with and without implantation of an abdominal wall patch (AWP) after laparotomy did not reach significance [4/68 patients with AWP (5.9%) vs. 31/200 patients without AWP (15.5%), $p = 0.06$].

Details regarding postoperative treatment are summarized in **Table 4**. Significant differences between patients with SBO and non-SBO were found for time to full enteral feeding ($p = 0.02$) and the duration of the chest tube insertion ($p = 0.02$). We identified a significant correlation between the duration of the chest tube insertion and the corresponding findings ($p < 0.0001$), whereby chylothorax showed the longest duration with a median of 10 days. Also, the duration of the chest tube insertion among chylothorax was longer in patients with SBO than in patients with non-SBO (median 17 days vs. 10 days; $p = 0.23$). Chylothorax was found to be predominant (17/24, 70.8%) when focusing on the findings beyond the cut-off value of 16 days. In addition, 4 of 17 (23.5%) patients with SBO compared to 13 of 139 (9.4%) patients without SBO had a chest tube for chylothorax beyond 16 days, but this correlation did not reach statistical significance ($p = 0.09$).

Concerning re-operations during the observation period, SBO occurred in two of 91 patients (2.2%), who underwent one or more surgical procedures other than for SBO. In contrast, 36 of 246 patients (14.6%) without re-operation developed SBO ($p = 0.001$). No effect of timing on the re-operation could be identified (**Table 4**).

Logistic Regression and Multivariate Analysis

The statistically significant effects of the surgical approach, the duration of chest tube insertion, and re-operation on SBO could be confirmed by further analysis and proved to be independent predictors (increase duration of chest tube insertion by 3 days: OR 1.33; 95% CI 1.10–1.60; $p = 0.003$; no re-operation: OR 19.9; 95% CI 2.28–174.24; $p = 0.01$). Midline laparotomy in comparison to thoracoscopy significantly increased the relative risk of SBO 3-fold (RR 3.00, 95% CI 0.95–9.48; $p = 0.04$). In contrast, performing one or more re-operations had a protective effect and reduced the risk of SBO by 85% (RR 0.15; 95% CI 0.04–0.61; $p = 0.001$). The likelihood of SBO was 14.6% in patients without re-operation, decreased to 1.6% with one re-operation, and increased slightly to 5.3% with two re-operations.

In the attempt to identify risk factors for the formation of adhesions, further analysis of 17 ASBO patients in comparison to 299 patients with non-SBO was performed.

Adhesive Small Bowel Obstruction

Focusing on adhesion formation, which caused 17 of 28 SBOs with available surgical reports (60.7%), we found significant predictors for ASBO. No significant difference concerning demographics, patient characteristics, and preoperative treatment could be detected (**Table 5**). Regarding surgical characteristics and intraoperative findings (**Table 6**), all 17 patients with ASBO received a midline laparotomy, whereas no child with thoracoscopy developed ASBO. Therefore, we determined that midline laparotomy significantly increased the risk of developing ASBO by 28% (95% CI 1.20–1.36; $p = 0.03$). Defect size also showed a significant difference with predominantly larger defect sizes in patients with ASBO. In logistic regression analysis, this did not show an effect. With regard to postoperative treatment, the time to full enteral feeding was significantly longer in patients with ASBO but could not be confirmed by logistic regression analysis (**Table 7**). In accordance with our previous findings concerning SBO in general, there was a correlation between the duration of the chest tube insertion for serous effusion and chylothorax with a longer duration in our ASBO cohort as compared to patients with non-SBO. After a cut-off value of 16 days, this difference was significant for serous effusion ($p = 0.04$), but not for chylothorax ($p = 0.31$). Due to the small number of patients, no further statistical analysis could be performed (**Table 8**). A significantly lower incidence of ASBO was observed in patients, who underwent secondary surgeries during follow-up, with a trend concerning excision of the abdominal wall patch (**Table 9**). The duration of the chest tube insertion also significantly increased the risk for ASBO (increase duration by 3 days: OR 1.22; 95% CI 1.01–1.46; $p = 0.04$), while re-operations were associated with a significantly decreased risk of ASBO (one or more re-operations: RR 0.16; 95% CI 0.02–1.17; $p = 0.049$) (**Table 10**). Both factors were found to be independent predictors in multivariate analysis (no re-operation: OR 10.05; 95% CI 1.13–89.30; $p = 0.04$) (**Table 11**). Also, with the increasing duration of chest tube insertion, the probability of ASBO was higher in patients without re-operation than in patients with one or more re-operations (**Table 12**).

TABLE 3 | Differences in surgical characteristics and intraoperative findings between patients with SBO and non-SBO.

	SBO ***n* = 38**	**Non-SBO** ***n* = 299**	***p*-value**
Timing of reconstruction in days	4.5 (0–18)	6 (0–21)[a]	0.42
Median (min.-max.)			
Surgical time in minutes – median (min.-max.)	173 (95-283)[b]	174 (58–388)[c]	0.82
Operation at neonatal intensive care unit	24 (63.2)	178 (59.5)	0.67
Surgical approach – n (%)			
Minimally invasive	3 (7.9)	66 (22.1)	**0.04**
Open	35 (92.1)	233 (77.9)	
Side of defect – n (%)			
Left side	36 (94.7)	284 (95)	0.15
Right side	2 (5.3)	49 (16.4)	
Bilateral	0 (0.0)	2 (0.7)	0.54
Liver-up in left sided CDH– n (%)	22 (61.1)	138 (48.6)	
Hernia type – n (%)			
Discontinuity/without hernia sac	32 (84.2)	260 (87)	0.64
with hernia sac	6 (15.8)	39 (13)	
Defect size[d] – n (%)			
A	5 (13.1)	26 (8.7)	0.25
B	11 (28.9)	104 (34.8)	
C	21 (55.3)	137 (45.8)	
D	1 (2.6)	32 (10.7)	
Anatomic localisation of the defect – n (%)			
Bochdalek	35 (94.6)[e]	276 (92.6)[f]	1.00
Morgagni or Larrey	2 (5.3)	22 (7.4)	
Reconstruction of the diaphragm – n (%)			
Primary closure	6 (15.8)	59 (19.7)	0.56
Patch correction	32 (84.2)	240 (80.3)	
Abdominal wall closure with patch – n (%)	4 (10.5)	64 (21.4)	0.12
Intraoperative adhesion prevention– n (%)			
Seprafilm®	0 (0.0)	7 (2.3)	1.00
Fibrin	0 (0.0)	2 (0.7)	1.00
Contamination class[g] – n (%)			
1	35 (94.6)	279 (93.3)	0.77
2	3 (5.4)	19 (6.4)	
3	0 (0.0)	1 (0.3)	
Cases with additional operative procedures – n (%)[h]	13 (34.2)	95 (31.8)	0.76
- Release of duodenal kinking	4 (10.5)	34 (11.4)	1.00
- Resection of meckel's diverticulum	2 (5.3)	16 (5.4)	1.00
- Resection of accessory spleen	3 (7.9)	15 (5.0)	0.44
- Hiatoplasty and fundoplication for GER	3 (7.9)	13 (4.3)	0.41
- Resection of ladd's bands	1 (2.6)	12 (4.0)	1.00
- Resection of lung sequestration	2 (5.3)	11 (3.7)	0.65
- Resection of accessory liver	0 (0.0)	12 (4.0)	0.37
- Miscellaneous additional procedures[i]	2 (5.3)	8 (2.7)	0.31
- Insertion of stoma	0 (0.0)	2 (0.7)	1.00

SBO, small bowel obstruction; non-SBO, no small bowel obstruction; CDH, congenital diaphragmatic hernia; GER, gastroesophageal reflux.

[a] *1 missing data.*

[b] *1 missing data.*

[c] *2 missing data.*

[d] *classified by lally et al. (1).*

[e] *1 missing data.*

[f] *1 missing data.*

[g] *classified by the cdc: surgical wound classification grades i–iv (31).*

[h] *cases received more than one additional procedure.*

[i] *suture of intestinal perforation, resection of intestine, mesentery adaption, closure of tracheoesophageal fistula, lymph node resection, and suture of pericardial defect. Bold values are significant.*

TABLE 4 | Differences in postoperative treatment and concerning re-operations between SBO- and non-SBO-patients.

	SBO ***n* = 38**	**Non-SBO** ***n* = 299**	**p-value**
Duration of invasive ventilation in days	22.5 (6–157)	21.0 (1–143)[a]	0.71
Median (min.-max.)			
Duration of NIV in days – median (min.-max.)	10.5 (0-147)	7 (0-176)[b]	0.40
Postoperative nutrition – n (%)			
Breast milk	18 (47.4)	136 (46.1)[c]	0.79
Formula	6 (15.8)	60 (20.3)	
Breast milk and formula	14 (36.8)	99 (33.6)	
Timing of postoperative oral nutrition in days	4 (1–12)	4 (1–49)[d]	0.62
– Median (min.-max.)			
Time to full enteral feeding in days	30 (9–99)[e]	23 (3–194)[f]	**0.02**
– Median (min.-max.)			
Insertion of chest tube – n (%)			
Intraoperative/preventive	1 (2.6)	10 (3.3)	0.95
Secondary	16 (42.1)	130 (43.5)	
Findings of chest tube – n (%)			
Serous effusion	9 (53.0)	65 (46.8)	1.00
Chylothorax	7 (41.2)	56 (40.3)	
Pneumothorax	1 (5.9)	14 (10.1)	
No findings	0 (0.0)	3 (2.2)[7]	
Empyema	0 (0.0)	1 (0.7)	
Duration of chest tube insertion in days	11 (4–39)	7 (1–39)[h]	**0.02**
– Median (min.–max.)			
Duration of antibiotic therapy in days	33 (7-114)[j]	30.0 (4-174)[j]	0.71
– Median (min.-max.)			
Escalation of antibiotic therapy – n (%)	24 (63.2)	169 (57.3)[k]	0.49
time to discharge in days – median (min.-max.)	61.5 (7–210)	53 (1–270)[l]	0.32
Number of re-operations – n (%)			
0	36 (94.7)	210 (70.2)	**0.03**
1	1 (2.6)	61 (20.4)	**0.001**
2	1 (2.6)	18 (6.0)	
3	0 (0.0)	8 (2.7)	
7	0 (0.0)	1 (0.3)	
8	0 (0.0)	1 (0.3)	
≥1	2 (5.3)	89 (29.8)	
timing to 1st re-operation in days	281 (1–561)	156 (12–1972)	0.61
– median (min.-max.)			
timing to 2nd re-operation in days	392 (-)	468.5 (39-3193)	0.90
— median (min.-max.)			
timing to 3rd re-operation in days	-	691.5 (185-1942)	-
— median (min.-max.)			

SBO, small bowel obstruction; non-SBO, no small bowel obstruction; NIV, non-invasive ventilation. Bold values are significant.

[a] *2 data missing.*
[b] *4 data missing.*
[c] *4 data missing.*
[d] *7 data missing.*
[e] *5 data missing.*
[f] *18 data missing.*
[g] *1 data missing.*
[h] *1 data missing.*
[i] *1 data missing.*
[j] *6 data missing.*
[k] *4 data missing.*
[l] *1 data missing.*

TABLE 5 | Differences in demographics, patient characteristics, and preoperative treatment between patients with ASBO- and non-SBO.

	ASBO ***n* = 17**	**Non-SBO** ***n* = 299**	***p*-value**
Follow-up in years — median (min.-max.)	4.2 (0.4–6.3)	4.1 (1.0–10.6)	0.86
Sex – n (%)			
Male	5 (29.4)	127 (42.5)	0.29
Female	12 (70.6)	172 (57.5)	
Birth mode – n (%)			
Vaginal	3 (17.7)	68 (23.6)[a]	0.77
Caesarean section	14 (82.3)	220 (76.4)	
Date of delivery in gw	37+6	38+0	0.82
– Median (min.-max.)	(35+0 – 41+5) [b]	(27+3 – 41+4)[c]	
Amnion infection syndrome – n (%)	0 (0.0)	6 (2.0)[d]	1.00
Birth weight in kg – median (min.-max.)	2.9 (1.9-3.9)[e]	3.0 (1.4-4.6)[f]	0.94
Birth hight in cm – median (min.-max.)	49.0 (40.0-55.5)[g]	50.0 (40.0-63.0)[h]	0.41
Outborn – n (%)	2 (11.8)	48 (16.1)	1.00
Associated structural malformations or syndromes – n (%)[i]	10 (58.8)	140 (46.8)	0.34
Urinary and genital	5 (29.4)	47 (15.7)	0.17
Minor cardiovascular	2 (11.8)	37 (12.4)	1.00
Syndromes	2 (11.8)	14 (4.7)	0.21
Musculoskeletal	1 (5.9)	21 (7.0)	1.00
Malformation of the kidneys	1 (5.9)	20 (6.7)	1.00
Omphalocele/abdominal hernia	1 (5.9)	3 (1.0)	0.20
Major cardiovascular	0 (0.0)	16 (5.4)	1.00
Cerebral	0 (0.0)	13 (4.4)	1.00
Hepatobiliary	0 (0.0)	7 (2.3)	1.00
Esophageal atresia/-stenosis	0 (0.0)	2 (0.7)	1.00
Trachea-/bronchomalacia	0 (0.0)	3 (1.0)	1.00
Antibiotics since delivery – n (%)	17 (100.0)	286 (96.0)[j]	1.00
Use of FETO – n (%)	2 (11.8)	22 (7.5)[k]	0.63
Use of ECMO – n (%)	9 (52.9)	121 (40.5)	0.31
Duration of ECMO in days – Median (min.-max.)	12 (6–14)	9 (4-22)	0.22

ASBO, adhesive small bowel obstruction; non-SBO, no small bowel obstruction; gw, gestational week; FETO, fetal endoscopic tracheal occlusion; ECMO, extracorporeal membrane oxygenation.

[a] *11 data missing.*
[b] *1 data missing.*
[c] *11 data missing.*
[d] *3 data missing.*
[e] *1 data missing.*
[f] *6 data missing.*
[g] *3 data missing.*
[h] *27 data missing.*
[i] *cases had more than one associated anomaly.*
[j] *1 data missing.*
[k] *5 data missing.*

DISCUSSION

Our study seems to confirm that SBO represents an important cause of morbidity after neonatal repair of CDH. We determined an incidence of 11.3% during a prospective 10-year observation period of 337 CDH survivors and identified many different underlying causes. Furthermore, independent risk factors for developing ASBO could be identified: patients requiring a midline laparotomy for the reconstruction of CDH showed a higher risk than patients after minimally invasive repair. Also, the duration of the chest tube insertion was independently predictive of ASBO. In contrast, subsequent re-operations revealed an unexpected protective effect.

In general, literature reports focussing on SBO in children are scarce and only a few studies mention SBO as a complication after CDH repair. To make interpretation and comparison even more difficult, there is often no differentiation between SBO with a broad spectrum of possible causative conditions and ASBO. Mainly retrospective studies with small numbers of patients are available. These are difficult to compare due to the lack of standardized follow-up and varying length of follow-up and thus the true incidence of ASBO in patients with CDH is still

TABLE 6 | Differences in surgical characteristics and intraoperative findings between patients with ASBO and non-SBO.

	ASBO ***n* = 17**	**Non-SBO** ***n* = 299**	***p*-value**
Timing of reconstruction in days – Median (min.-max.)	4 (2–16)	6 (0–21)[a]	0.77
Surgical time in minutes – median (min.-max.)	181.5 (95–255)[b]	174 (58–388)[c]	0.83
Operation at neonatal intensive care	12 (70.6)	178 (59.5)	0.37
Surgical approach – n (%)			
Minimally invasive	0 (0.0)	66 (22.1)	**0.03**
Open	17 (100.0)	233 (77.9)	
Side of defect – n (%)			
Left side	15 (88.2)	248 (82.9)	1.00
Right side	2 (11.8)	49 (16.4)	
Bilateral	0 (0.0)	2 (0.7)	
Liver-up in left sided CDH– n (%)	9 (60.0)	138 (55.7)	0.74
Hernia type – n (%)			
Discontinuity/without hernia sac	15 (88.2)	260 (87)	1.00
With hernia sac	2 (11.8)	39 (13)	
Defect size[d] – n (%)			
A	2 (11.8)	26 (8.7)	**0.04**
B	2 (11.8)	104 (34.8)	
C	13 (76.5)	137 (45.8)	
D	0 (0.00)	32 (10.7)	
Anatomic localisation of the defect – n (%)			
Bochdalek	16 (100.0)[e] 0	276 (92.6)[f]	0.61
Morgagni or Larrey	(0.00)	22 (7.4)	
Reconstruction of the diaphragm – n (%)			
Primary closure	2 (11.8)	59 (19.7)	0.54
Patch correction	15 (88.2)	240 (80.3)	
Abdominal wall closure with patch in open surgery – n (%)	3 (17.6)	64 (27.5)	0.57
Intraoperative adhesion prevention– n (%)			
Seprafilm®	0 (0.0)	7 (2.3)	1.00
fibrin	0 (0.0)	2 (0.7)	1.00
Contamination class[g] – n (%)			
1	16 (94.1)	279 (93.3)	1.00
2	1 (5.9)	19 (6.4)	
3	0 (0.0)	1 (0.3)	
Cases with additional operative procedures – n (%)[h]	7 (41.2)	95 (31.8)	0.42
- Release of duodenal kinking	3 (17.7)	34 (11.4)	0.43
- Resection of Meckel's diverticulum	0 (0.0)	16 (5.4)	1.00
- Resection of accessory spleen	1 (5.9)	15 (5.0)	0.60
- Fundoplication as GER-prevention	2 (11.8)	13 (4.3)	0.19
- Resection of Ladd's bands	1 (5.9)	12 (4.0)	0.52
- Resection of lung sequestration	1 (5.9)	11 (3.7)	0.49
- Resection of accessory liver	0 (0.0)	12 (4.0)	1.00
- Miscellaneous additional procedures[i]	1 (5.9)	8 (2.7)	0.40
- Insertion of jejunal feeding tube/stoma	0 (0.0)	2 (0.7)	1.00

ASBO, adhesive small bowel obstruction; non-SBO, no small bowel obstruction. Bold values are significant.

[a] *1 missing data.*

[b] *1 missing data.*

[c] *2 missing data.*

[d] *classified by Lally et al. (1).*

[e] *1 missing data.*

[f] *1 missing data.*

[g] *classified by the CDC: Surgical Wound Classification Grades I–IV (31).*

[h] *cases received more than one additional procedure.*

[i] *suture of intestinal perforation, resection of intestine, mesentery adaption, closure of tracheoesophageal fistula, lymph node resection, and suture of pericardial defect.*

TABLE 7 | Differences in postoperative treatment between patients with ASBO and non-SBO.

	ASBO ***n* = 17**	**Non-SBO** ***n* = 299**	***p*-value**
Duration of invasive ventilation in days – Median (min.-max.)	27.0 (7–138)	21.0 (1–143)[a]	0.35
Duration of NIV in days – median (min.-max.)	10.5 (0–147)	7 (0–176)[b]	0.12
Postoperative nutrition – n (%)			
Breast milk	8 (47.1)	136 (46.1)[c]	0.96
Formula	3 (17.7)	60 (20.3)	
Breast milk and formula	6 (35.3)	99 (33.6)	
timing of postoperative oral nutrition in days – Median (min.-max.)	4 (1–12)	4 (1–49)[d]	0.81
Time to full enteral feeding in days – median (min–max.)	33 (12–70)[e]	23 (3–194)[f]	**0.02**
Insertion of chest tube – n (%)			
Intraoperative/preventive	1 (5.9)	10 (3.3)	0.59
Secondary	9 (52.9)	130 (43.5)	
Findings of chest tube – n (%)	10	139[g]	
Serous effusion	6 (60.0)	65 (46.8)	0.85
Chylothorax	3 (30.0)	56 (40.3)	
pneumothorax	1 (10.0)	14 (10.1)	
no findings	0 (0.0)	3 (2.2)	
empyema	0 (0.0)	1 (0.7)	
duration of chest tube insertion in days – median (min.-max.)	12.5 (4–39)	7 (1-39)[h]	0.13
findings of second chest tube – n (%)	0	11	
serous effusion	0 (0.0)	4 (36.4)	1.00 -
pneumothorax	0 (0.0)	4 (36.4)	
chylothorax	0 (0.0)	3 (27.3)	
empyema	-	-	
duration of antibiotic therapy in days – median (min.-max.)	39 (10–114)[i]	30.0 (4–174)[j]	0.30
escalation of antibiotic therapy – n (%)	11 (64.7)	169 (57.3)[k]	0.55
time to discharge in days – median (min–max.)	63 (25–184)	53 (1–270)[l]	0.33

ASBO, adhesive small bowel obstruction; non=SBO, no small bowel obstruction; NIV, non-invasive ventilation. Bold values are significant.
[a] *2 data missing.*
[b] *4 data missing.*
[c] *4 data missing.*
[d] *7 data missing.*
[e] *1 data missing.*
[f] *18 data missing.*
[g] *1 data missing.*
[h] *1 data missing.*
[i] *1 data missing.*
[j] *6 data missing.*
[k] *4 data missing.*
[l] *1 data missing.*

unknown. Identification of risk factors is impaired for the same reasons. Literature reports an incidence of SBO from 3 to 20% in patients with CDH who have a wide range of follow-up periods (32). However, the incidence of postoperative bowel obstruction in this specific population seems to be considerably higher than in neonates undergoing laparotomy other than for CDH (19, 20).

Symptoms of SBO were caused by a variety of underlying conditions in our cohort as follows: adhesive bands > volvulus / duodenal kinking > inner herniation / CDH-recurrence > Ladd's bands. Similar findings were observed by Jancelewicz et al. in a prospective follow-up study of 99 CDH survivors: adhesions in 54%, recurrence in 39%, and volvulus in 8% (33). Literature states that CDH predisposes to volvulus, which was the second most frequent cause of SBO, and it occurred in 1.4% of all participants in our study. This data correlate with other reports that identified an incidence of 0.3–1.0% (27, 33). Interestingly, Ward et al. found that the prophylactic Ladd procedure, which was assumed to prevent developing volvulus, was associated with a 3-fold increased risk of subsequent volvulus (27). Furthermore, a higher

TABLE 8 | Correlation between findings of pleural effusion and duration of chest tube insertion.

	ASBO ***n* = 10**	**Non-SBO** ***n* = 139**[a]	***p*-value**
Duration of chest tube insertion in correlation to findings in days – median (min.-max.)			
chylothorax	17 (6–20)	10 (2–39)	
empyema	-	9 (-)	
serous effusion	7.5 (4–39)	6 (1–25)	
pneumothorax	25 (-)	5.5 (1–31)	
findings among chest tube with duration > 16 days – n (%)	5 (50.0)	17 (12.3)	**0.01**
chylothorax	2 (20.0)	13 (9.4)	0.27
serous effusion	2 (20.0)	3 (2.2)	**0.04**
pneumothorax	1 (10.0)	1 (0.7)	0.13

ASBO, adhesive small bowel obstruction; non-SBO, no small bowel obstruction. Bold values are significant.
[a] *1 data missing.*

TABLE 9 | Re-operations during follow-up concerning patients with ASBO and non-SBO.

	ASBO ***n* = 17**	**Non-SBO** ***n* = 299**	***p*-value**
Number of re-operations – n (%)			
0	16 (94.1)	210 (70.2)	0.21
1	0 (0.0)	61 (20.4)	
2	1 (5.9)	18 (6.0)	
3	0 (0.0)	8 (2.7)	
7	0 (0.0)	1 (0.3)	
8	0 (0.0)	1 (0.3)	
≥1	1 (5.9)	89 (29.8)	**0.049**
Timing to 1st re-operation in days – Median (min.-max.)	1 (-)	156 (12–1972)	0.09
Timing to 2nd re-operation in days — Median (min.-max.)	392 (-)	468.5 (39–3193)	0.90
Timing to 3rd re-operation in days — Median (min.-max.)	-	691.5 (185–1,942)	-
Procedure			
- excision of abdominal wall patch[a] – n (%)	1 (33.3)	55 (85.9)	0.07
- change of abdominal wall patch[a] – n (%)	1 (33.3)	6 (9.4)	0.29
- recurrence – n (%)	0 (0.0)	31 (10.4)	0.39
- other intestinal procedure[b] – n (%)	0 (0.0)	28 (9.4)	0.38
- hiatoplasty and fundoplication – n (%)	0 (0.0)	22 (7.4)	0.62
intraoperative adhesion prevention – n (%)	0 (0.0)	16 (5.4)	1.00

ASBO, adhesive small bowel obstruction; non-SBO, no small bowel obstruction. Bold values are significant.
[a] *percentage referred to patients with abdominal wall patch (ASBO: n = 3, non-SBO: n = 64).*
[b] *resection of Meckel's diverticulum, resection of intestine/stoma, insertion of jejunal feeding tube, pyloromyotomy.*

incidence of SBO was found following surgical interventions for malrotation and of the upper gastrointestinal tract (15, 16). Even though Ladd's procedure is considered routine during CDH repair in many centers, it might be questionable after these findings.

Adhesion Formation

Adhesions are initially a normal step of the repair mechanisms after peritoneal damage, but an imbalance among fibrinolysis, fibrin formation, coagulation, and inflammation results in persistent fibrous bands (7, 34, 35). The pathophysiological processes behind adhesive formations are still the subject of current research. After a peritoneal injury, a fibrinous exudate is formed as the first step. The formation of fibrin is the result of the coagulation cascade, which can be initiated by tissue factors. On the other hand, fibrinolysis activated by plasmin induces fibrin degradation and is enabled by tissue plasminogen activator (tPA) and urokinase-like plasminogen activator (uPA). Through this cascade, the fibrinous formations should be resorbed within days. Tissue factors as well as tPA, uPA. and their inhibitor, plasminogen activator inhibitors group 1 (PAI-1) are expressed by the mesothelial cells of the peritoneum, while inflammatory

TABLE 10 | Predictors of adhesive small bowel obstruction (ASBO) using univariate analysis and logistic regression.

	RR/OR (95% CI)	*p*-value
defect size B vs. A	0.25 (0.03–1.86)	0.18
defect size C vs. A	1.23 (0.26–5.80)	0.79
surgical approach (midline laparotomy)	1.28 (1.20–1.36)[a]	**0.03**
≥1 re-operations	0.16 (0.02–1.17)	**0.049**
excision of abdominal wall patch	0.10 (0.01–0.99)	0.07
time to full enteral feeding in days (+1)	1.01 (1.00–1.03)	0.11
duration of chest tube insertion in days (+3)	1.22 (1.01–1.46)	**0.04**

ASBO, adhesive small bowel obstruction; RR, relative risk; OR, odds ratio; CI, confidence interval. Bold values are significant.

[a]*RR could not be calculated because all patients with ASBO underwent midline laparotomy. Therefore, column risk was determined: (17/17) MIS / (233/299) median laparotomy = 1.28.*

TABLE 11 | Independent predictors of adhesive small bowel obstruction (ASBO) using multivariate analysis.

	OR (95% CI)	*p*-value
No re-operation vs. ≥1 re-operations	10.05 (1.13–89.30)	**0.04**
duration of chest tube insertion in days (+3)	1.29 (1.06–1.58)	**0.01**

ASBO, adhesive small bowel obstruction; OR, odds ratio; CI, confidence interval. Bold values are significant.

TABLE 12 | Likelihood of adhesive small bowel obstruction (ASBO) as a function of the duration of chest tube insertion and the number of re-operations.

	Likelihood in %, no re-operation	Likelihood in %, ≥1 re-operation
Duration of chest tube insertion in days		
0	4.39	0.46
2	5.17	0.54
4	6.08	0.64
6	7.14	0.76
8	8.37	0.90
10	9.78	1.07
12	11.41	1.27
14	13.26	1.50
16	15.37	1.77
18	17.74	2.10
20	20.38	2.48
22	23.31	2.94
24	26.52	3.47
26	30.00	4.09
28	33.73	4.82
30	37.67	5.67

ASBO, adhesive small bowel obstruction.

cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α), cause an imbalance with a tendency to fibrin deposition (7, 35). If it remains for too long, the fibrin becomes organized into fibrous strands or membranes, consisting of collagen, blood vessels, and nerves (35).

In addition to cytokines and other mediators, the cellular response to injury may also play a role. Recruitment of neutrophils, as the main subgroup of leukocytes, is the first response to trauma. Neutrophils form neutrophil extracellular traps (NETs) and their influence has been described in various pathologies (36, 37). The NETs are also able to modulate the immune response to support inflammatory processes. Therefore, high levels of NETs have been found in the adhesive tissue.

Further influencing factors expose neonates and especially those with pulmonary hypertension to a greater risk of developing adhesions after laparotomy (8, 9). Peritonitis has been described as a possible risk factor for the formation of adhesions (6). Accordingly, in patients with neonatal laparotomy due to inflammatory conditions like necrotizing enterocolitis, a predominance of dense adhesions has been discovered during re-laparotomy for SBO (15).

In our cohort, CDH repair was performed with no contamination in the vast majority of patients so an additive effect of peritoneal inflammation on the formation of adhesions seems less likely. The majority received antibiotic treatment directly after birth. Laboratory parameters of infection or inflammation were not collected in our study, only escalation of antibiotic therapy was evaluated, and it did not differ between patients with SBO and non-SBO. However, this parameter is not a sufficient surrogate parameter for a proinflammatory status. So far, there are no further studies in patients with CDH reporting any of these conditions in correlation with ASBO.

The severity of adhesions was not addressed by our data, due to a lack of a standardized classification system. Coccolini et al. suggested a regimented classification system for adhesions - the peritoneal adhesion index (PAI) - in an effort to standardize their definition and subsequent analysis (38). A survey on its feasibility showed a high acceptance among surgeons (39). In a prospective observational study of postoperative ASBO, Sisodia et al. found that PAI is a sensitive and effective tool for the quantitative assessment of intraabdominal adhesions (40). In addition, PAI has already been used as a variable in several studies (41, 42). Its implementation could provide further information on risk factors and their influence by making it easier to compare the results of different centers.

No improvement concerning the incidence of ASBO after laparotomy in childhood can be observed (16). Besides general surgical principles with minimal and careful handling of the bowel, minimizing the blood loss, and avoiding devascularization and desiccation of the bowel during surgery, other therapeutic strategies based on a better understanding of the underlying pathophysiology of the formation of adhesions should be considered. To achieve the separation of damaged surfaces (35), adhesion barriers, such as the hyaluronic acid-carboxymethylcellulose membrane Seprafilm®, showed a reduced severity of adhesions as well as few abdominal complaints in adults (43). Other adjuncts based on the current

research may be introduced in the near future. It has been reported that DNases can dissolve NETs. Accordingly, Heuer et al. observed a significantly reduced formation of NETs in mice treated with DNase1 (44). In addition, DNase Knockout-mice with laparotomy-induced adhesions showed higher levels of NETs and increased adhesion formation, based on the experiments from Boettcher et al., suggesting an important role of DNases in this context. Furthermore, treatment with DNases in Wildtype-mice resulted in a significant reduction in laparotomy-induced adhesions without negatively affecting wound healing. The preprint may be found on Research Square (https://doi.org/10.21203/rs.3.rs-1077792/v1).

In the following section, the risk of ASBO in CDH is critically reviewed in the context of the current literature regarding the potential and proven influencing factors and our findings are discussed accordingly.

Minimally Invasive Surgery vs. Open Surgery

A significantly increased rate of ASBO after open abdominal reconstruction of the diaphragm was expectedly replicated in our study with an incidence of 6.8% among patients with midline laparotomy and 0% in patients with a minimally invasive approach. The CDH Study Group revealed similar results already for the initial hospital stay. They showed that patients, who underwent MIS, had a five times lower risk of ASBO requiring an operation until discharge than patients with OS (OR 0.19; 95% CI 0.06–0.60, $p = 0.005$). Also in defect size A, a significantly lower risk of ASBO in patients who underwent MIS could be detected, while it was not significant for other defect sizes (45). In contrast to the CDH study group, we did not observe any ASBO after minimal-invasive repair in the long term, but in a smaller cohort.

Thoracoscopy for CDH repair has become the more popular minimally invasive approach than laparoscopy. Only very few studies report on long-term data regarding ASBO in relation to the surgical approach and MIS cohorts are often too small to draw any conclusions (24). Similar to our findings, Jancelewicz (2010) noticed symptoms of SBO only in correlation with CDH recurrence in the MIS cohort and there was no SBO due to adhesions detected (46).

However, not every patient is suitable for minimal-invasive CDH repair. Thoracoscopy poses a greater risk of technical difficulties leading to conversion as well as intraoperative hypercapnia and acidosis, which potentially affects neurological development (47). Also, several studies described significantly higher recurrence rates after thoracoscopic repair of the diaphragm (45, 48). In our minimal invasive cohort, only patients with CDH recurrence (4%) presented with signs of SBO and no ASBO was encountered. Even in patients with thoracoscopic implantation of a diaphragmatic GoreTex® patch (30%), no problems due to ASBO were observed. This might be due to the less-invasive access itself and pleural rather than peritoneal trauma in thoracoscopy. Therefore, in the subset of patients with CDH having smaller defect sizes (A and B), the thoracoscopic approach with meticulous surgical technique to reduce the risk of CDH recurrence may be superior to the open approach with regard to the prevention of adhesive SBO.

Defect Size

So far, there is only one study reporting on the incidence of SBO in correlation to defect size. Putnam et al. recognized an increasing incidence of of ASBO with larger defect-size during the initial hospital stay, with a significantly higher incidence after open abdominal surgery. In open surgery, the incidence in correlation to defect size was as follows: A, 2.3%; B, 2.7%; C, 6.6%; and D, 7.9% (45). In our longitudinal follow-up, we found the following incidences of ASBO in open surgery: A, 16.7%; B, 2.9%; C, 8.5%; and D, 0%. The subgroups with defect sizes A and D were small. Within the larger subgroups with defect sizes B and C, the difference was not statistically significant ($p = 0.24$). Thus, larger observational cohort studies with longitudinal follow-up will have to be awaited to verify, if defect size by itself or other factors like patch material and surgical access have an impact on the development of adhesive SBO.

Patch Repair for Diaphragmatic and Abdominal Wall Reconstruction

A metaanalysis of 10 studies and 1,273 patients reported an about twice higher risk of SBO for patients with patch implantation compared to patients with primary closure of the diaphragm (OR 1.90; 95% CI 1.31–2.76). The incidence of SBO was 6.6% after primary repair and 12% after patch repair. There was no differentiation of ABSO or analysis according to different patch materials, but mainly PTFE was used (32). This makes an interpretation difficult since SBO can have multiple different causes as explained above.

In our cohort, the overall incidence of ASBO was only 6.3% in OS with 11.8% after primary repair and 6% after implantation of a diaphragmatic patch. The difference was not significant due to the small number of patients with primary repair in OS. There was also no difference in the high patch rate in patients with ASBO and non-ASBO (ASBO/non-ASBO: 88.2% vs. 94% patch; $p = 0.3$). The high number of patients with large defects in OS (C: 57.1%; D: 12.3%) and therefore requiring a diaphragmatic patch reflects disease-severity being an ECMO center. The majority of patients with a primary repair was operated by minimal-invasive access at our center (73.8%), which is a potential bias. No ASBO was observed in our patients with MIS despite a patch rate of 30%. In contrast to the results of the metaanalysis, the GoreTex® patch by itself might not contribute to a higher risk of ASBO, since we were using the same patch material irrespective of surgical access.

There was also no difference regarding the incidence of ASBO in correlation to the implantation of an abdominal wall patch: about 4.4% in patients with and 7% in patients without an abdominal wall patch (AWP). The rate of an AWP did not differ between both subgroups (AWP: 17.6% ASBO vs. 27.5% non-SBO; $p = 0.57$). To date, there are no further studies explicitly mentioning abdominal wall patches in correlation with ASBO in patients with CDH. It has to be considered that the abdominal wall patch is a surrogate parameter for the severity of CDH. Mostly, severely affected neonates are referred for

treatment to ECMO-centers which explains the high rate of AWP implantation in our cohort. Other centers might therefore find a difference concerning this parameter. Nevertheless, we detected a comparatively low incidence of ASBO. Interestingly, a higher incidence of ASBO was shown for neonates with abdominal wall defects (25% in 59 patients with gastroschisis vs. 13% in 111 patients with omphalocele, $p = 0.06$). Only seven patients received a prosthetic mesh in this cohort. In multivariate analysis, sepsis and fascia dehiscence were identified as independent predictors (18). Thus, the implantation of an AWP (GoreTex®) in severely affected neonates with CDH and a hypoplastic abdominal cavity may be more favorable than only skin closure with iatrogenic fascia dehiscence concerning the development of ASBO.

Patch Material

Even though the lower incidence of ASBO in patients with implantation of a diaphragmatic or abdominal wall GoreTex® patch was not significant, there might still be something about it to be considered. Literature also gives hints to differences in adhesion formation in correlation to patch material. Patients, who received absorbable biosynthethic patches like Surgisis-Gold® (SIS) or AlloDerm® developed a higher rate of SBO than patients with a nonabsorbable mesh like Dacron® or GoreTex® in some studies, but subgroups of patch patients were small (33, 49, 50). Jancelewicz et al. identified SIS as the only significant subtype in univariate logistic regression analysis comparing primary and patch repair (SIS vs. GoreTex® vs. SIS+GoreTex®) with an OR of 8.1 (95% CI 2-28; p = 0.001) (33). On the other hand, no significant difference concerning ASBO between SIS (7%) and GoreTex® (4%) was observed in a retrospective study of 72 patch patients with a minimal follow-up of 30 days (51).

A less adhesive effect of GoreTex® has been reported in 91% of adults, who had undergone laparoscopic ventral incisional hernia repair with GoreTex® mesh implantation. Adhesions were either not present or were filmy and avascular at the timing of re-operation (52).

Furthermore, polytetrafluoroethylene (PTFE), also used for GoreTex®, was studied as a barrier to prevent adhesions in an animal model and was implanted to cover the injured peritoneum after pelvic surgery. The extent of adhesions was significantly less, and fewer animals had adherent intestinal loops compared to a control group, indicating an effective adhesion prevention barrier (53). Therefore, the type of mesh used for the diaphragmatic reconstruction may influence the development of adhesions and consequently ASBO. We hypothesize that the use of GoreTex®-patches may reduce intraabdominal adhesions due to their specific content of expanded PTFE. This would be an added advantage for the use of GoreTex® for diaphragmatic patch repair besides the seemingly lower long-term recurrence rate (54).

Laparotomy

Furthermore, the way of abdominal access may influence the development of ASBO. Recently, Janssen et al. revealed an incidence of SBO after CDH repair of 20% in 112 patients but did not differentiate for ASBO. Compared to our overall SBO rate of 11.3% in 337 patients, this was significantly higher ($p = 0.04$). The SBO occurred in 19% of 98 patients after subcostal laparotomy and in 21% of 14 patients with either thoracoscopy, thoracotomy, or laparoscopy. The risk of SBO was significantly higher after patch repair in 35 patients (OR 3.5, 95% CI 1.2–10.0) (24). In their study cohort, a higher proportion of open abdominal reconstruction of the diaphragm was performed as compared to our study cohort, but this difference is not statistically significant (87.5% vs. 79.5%; $p = 0.07$). On the other hand, the patch-rate was significantly lower in their cohort (31% vs. 80.7%; $p < 0.00001$). Yokota et al. noticed an intestinal adhesion obstruction of 17.6% in 74 CDH survivors with subcostal laparotomy and 6.7% in 240 patients with other neonatal laparotomies than for CDH ($p = 0.023$) (23). We encountered a lower incidence of 6.3% for ASBO among 268 patients with CDH who underwent midline laparotomy. There is a significant difference between both cohorts: the incidence of ASBO was higher after subcostal laparotomy ($p < 0.005$) despite a lower rate of patch repair (33.8% vs. 93.7%; $p < 0.00001$).

Interestingly, both authors reported a similar patch rate of about 30% and an incidence of (A)SBO of nearly 20% after subcostal laparotomy, whereas a significantly higher patch rate was observed with a significantly lower rate of (A)SBO following the median laparotomy in our cohort. We had a comparable patch rate of 30% in our MIS cohort, which was not associated with ASBO. Since in all these cohorts, solely Goretex® was used as a patch material, this might not affect the rate of ASBO by itself. While it is well-known that postoperative intestinal obstruction is reduced after laparoscopy compared to laparotomy (55), possibly the difference in abdominal access (subcostal vs. midline laparotomy) might also play a role in the development of ASBO that has been neither reported nor investigated so far. An explanation for this finding could be that with subcostal incisions, the abdominal wall muscles have to be divided, whereas these are kept intact using the midline laparotomy. The more invasive abdominal access may contribute to a more intense or longer activation of the healing cascade possibly resulting in the development of more adhesions, especially in neonates with pulmonary hypertension as explained in detail above.

ECMO Therapy

To our knowledge, there is only one study explicitly reporting on SBO in correlation to ECMO therapy in patients with CDH. A protective effect of ECMO treatment was described with a significantly reduced rate of SBO of 9% in 22 patients with ECMO compared to 22% in 90 patients without ECMO (OR adjusted 0.2; 95% CI 0.0–1.0; $p < 0.05$) with no specification of the underlying cause of SBO (24). These findings could not be confirmed in our larger cohort regarding ASBO: a lower incidence was detected irrespective of ECMO therapy (6.9% in 130 patients with ECMO vs. 4.3% in 186 patients without ECMO, $p = 0.32$). Albeit a significant difference in ECMO treatment between both study cohorts (Janssen 19.6% vs. our cohort 41.1%; $p < 0.0001$), which may also have attributed to the different results. Possibly, the more relevant difference between both study cohorts is the timing of CDH repair: While Janssen et al. routinely

perform CDH repair under ECMO therapy, we prefer to operate after weaning off ECMO. Therefore, alterations of the healing cascade, coagulation, and immune system due to using an ECMO circuit with cannulas, tubes, pumps, and blood from donors as well as specific drug administration and heparinization under ECMO therapy might play a role in the formation of adhesions and thereby explain the different findings in these two cohorts. Further basic research is needed to elucidate the biochemical and cellular processes involved and larger cohort studies with longitudinal follow-up to verify these findings.

Time to Full Enteral Feeding

A correlation between time to enteral feeding and the occurrence of ASBO has been described in the literature. In neonates with spontaneous intestinal perforation, the duration of parenteral nutrition showed a significant effect on developing SBO later in life. However, a causal relationship was not confirmed by the authors but was considered to reflect the initial severity of the bowel disease (56). Regarding postoperative nutrition, neither the type nor the timing of postoperative oral feeding revealed a correlation with SBO in our cohort. Time to full enteral feeding showed a significantly longer time in the ASBO as compared to the non-SBO-group, but further analysis using logistic regression could not confirm these results. Therefore, it cannot be considered predictive of ASBO in our cohort. We started giving glucose on the first and breast milk or formula on the second postoperative day. Time to full enteral nutrition may be prolonged in children with more severe lung hypoplasia and the need for prolonged ventilatory support with consequently a longer time of analgosedation. This medication may also influence intestinal peristalsis with reduced intestinal motility and gastroesophageal reflux delaying enteral feeding and prolonging the need for parenteral nutrition. Reduced intestinal peristalsis might contribute to the formation of more dense or extensive adhesions. In our cohort, we could not detect an influence of any of the above-mentioned parameters, which may be due to the overall CDH severity in our open surgery cohort and different at other centers.

Chest Tube

The duration of the chest tube insertion revealed a significant independent effect on the occurrence of SBO and ASBO. To the best of our knowledge, a correlation between the duration of the chest tube insertion and adhesive intestinal obstruction has not been described before. This circumstance may be explained in context with the specific population of patients with CDH, in which the separation between the thoracic and abdominal cavity is incomplete. Even after reconstruction, the diaphragm cannot be assumed impermeable, neither after primary nor after patch repair. Therefore, intrathoracic processes, such as chest tube insertion, could affect the abdominal environment. On the one hand, irritations of the tube induce a local inflammatory response and on the other hand, the injury of the pleura activates or prolongs physiological tissue repair processes (57).

Considering the correlation between the duration of the chest tube insertion and the type of pleural effusion, the influence of serous effusion or chylothorax or rather their consequences also have to be considered. Loss of chyle and its components, especially chylomicrons, proteins, and lymphocytes, lead to malnutrition, increased risk of thrombosis, and secondary immunodeficiency (58). In addition to the influence on the immune system, a procoagulant effect may also play a role in the formation of adhesions in the presence of chylothorax and substitution with fresh frozen plasma. An impaired flow of abdominal chyle might also be a causative factor for adhesion formation. Also, serous effusion is due to pleural and/or peritoneal injury and contains the so-called "reactive" mesothelial cells, macrophages and blood-derived cells like lymphocytes, and neutrophil granulocytes among others. "Reactive" mesothelial cells also display phagocytic activity. As has been explained above, these cells, tissue-factors, and inflammatory cytokines are involved in the pathogenesis of adhesion formation (7, 44). This might explain the correlation between serous effusion and chylothorax observed in our ASBO cohort, despite lacking significance for chylothorax due to the small patient number.

Re-operations

In general, it is believed that repeated abdominal surgical interventions also trigger the formation of more adhesions. In children, this seems to be supported by a recurrence rate of ASBO from 0–29% (16). In a large study with a long follow-up of 500 adult patients with ASBO and also including those with surgical interventions in childhood, an increasing 10-year risk of obstruction was calculated in correlation with the number of episodes of ASBO: it was 18% after one episode and 63% after 4 episodes (59).

In contrast, re-operation was associated with a decreased rather than an increased risk of subsequent ASBO in our cohort. Yet, long-term follow-up has to be waited for the verification of these preliminary findings and final interpretation. In adults, similar findings were also reported. Patients with one previous surgery had more severe adhesions than patients with two surgeries, measured by the need for surgery for postoperative adhesive intestinal obstruction (40). Another study found that adults treated surgically for ASBO were less likely to have recurrent ASBO and the time to recurrence was longer. The authors indicated that in some of the patients treated surgically, the causative factor for adhesion formation was eliminated at the time of surgery (60). It must also be considered that in patients, who required laparotomy in the neonatal period, the incidence of SBO is higher than compared to older children (19, 22). This may be related to the reduced anti-inflammatory capacity and increased production of proinflammatory cytokines in neonates, which supports the persistence of adhesive formation, as described above (7–9). Our study participants, who underwent re-operation in the abdominal cavity other than for SBO, also received lysis of adhesions, and the timing of re-operation was mostly beyond the neonatal period. Presumably, a more mature immunological status at re-operation may lead to less adhesion formation and fewer sequelae.

Mortality and Morbidity

In a review on adhesions in children and adolescents, mortality resulting from ASBO has been found to be 0% to anecdotal 71%, but nowadays, it is still correlated to septicaemia or the underlying comorbidities (16). In patients with CDH, an overall late mortality of 5% has been observed mainly with gastrointestinal complications (14). Though we did not observe mortality in our patients with SBO, there might be hidden mortality: two patients deceased at an older age due to severe "gastroenteritis". Since no autopsy was performed, the real cause remains unknown but might as well have been due to either CDH recurrence with bowel incarceration or decompensated ASBO with consecutive septicaemia.

The need for bowel resection due to ASBO has been reported in 16% (17) and as much as 35% of patients after abdominal wall defects (18). In a large study of 414 neonates, intestinal perforation and gangrenous bowel were noted in 12.5 and 16.7%, respectively (15). Thus, the need for bowel resection in 17.6% of our patients is in accordance with the reports from the literature. Lautz et al. determined a delay of surgical intervention of >2 days after admission in patients without clinical improvement predictive of bowel ischemia and necrosis (61).

In children, a failure of conservative treatment of ASBO has been reported in 45–100%, while the majority of symptoms in adulthood resolve spontaneously, resulting in a much higher proportion of patients requiring relaparotomy in childhood (16). Accordingly, only three of our 38 patients with SBO (7.9%) could be managed conservatively in our cohort. These special findings should be considered in patients with CDH presenting with ASBO to reduce morbidity and mortality with a timely approach.

LIMITATIONS AND STRENGTHS

First, this is not a multicentre study, but the follow-up of a large monocentric patient cohort treated with standardized surgical techniques and prospective follow-up may also be considered a strength. The high number of patients participating in our follow-up program after discharge may be another strength allowing for reliable detection of the incidence of SBO and aiming at identifying possible risk factors. Yet, the impact of adhesions is under-reported because symptoms due to adhesions that did not result in SBO were not included. Furthermore, the number of patients with a minimal-invasive repair is lower than the number of patients with an open approach. Neither the comparison of ASBO rate depending on different patch materials was possible due to the sole use of GoreTex® in this cohort nor was the comparison depending on different abdominal approaches because being an ECMO Center, a median laparotomy is the preferred access in our center for severely affected CDH neonates necessitating the implantation of an AWP in a substantial subset of patients.

CONCLUSION

Symptoms of SBO are encountered with several underlying causes. Adhesive SBO was only observed after open CDH repair in our large cohort with prospective follow-up, which seems to underline the protective effect of MIS in a select subset of patients. In comparison to literature reports, a midline laparotomy might be associated with less ASBO than subcostal incisions. Furthermore, the implantation of Goretex® patches seems to be associated with less formation of adhesions, which is reflected by our comparatively low ASBO rate. Multivariate analysis revealed the duration of chest tube insertion as a risk factor and one or more re-operations as a protective factor for the occurrence of ASBO. Chest tube irritation over a prolonged period, possibly in combination with the cellular and immunologic consequences from serous effusion or chylothorax, may influence the occurrence of ASBO in patients with CDH during the neonatal period, reflecting an imbalance between anti- and proinflammatory responses. In the future, novel therapeutic strategies based on a better understanding of the biochemical and cellular processes involved in the pathophysiology of adhesion formation might contribute to a reduction of peritoneal adhesions and their associated morbidity and mortality.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because data was pseudonomized due to longitudinal follow-up and is saved in a local database. Requests to access the datasets should be directed to sylvia.buettner@medma.uni-heidelberg.de.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee II of the University of Heidelberg, Medical Faculty Mannheim. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

KZ and A-MF had full access to all the data in the study, take responsibility for the integrity of the data and the accuracy of the data analysis, study design, conduct, data collection, data analysis, and data interpretation, writing, and revision of this manuscript. TS, NR, MB, and LW were involved in the supervision of data collection, data interpretation, revision of the manuscript, and final approval. SB contributed to the statistical plan, data analysis, and critical revision of the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Lally KP, Lasky RE, Lally PA, Bagolan P, Davis CF, Frenckner BP, et al. Standardized reporting for congenital diaphragmatic hernia–an international consensus. *J Pediatr Surg.* (2013) 48:2408–15. doi: 10.1016/j.jpedsurg.2013.08.014
2. Peetsold MG, Heij HA, Kneepkens CM, Nagelkerke AF, Huisman J, Gemke RJ. The long-term follow-up of patients with a congenital diaphragmatic hernia: a broad spectrum of morbidity. *Pediatr Surg Int.* (2009) 25:1–17. doi: 10.1007/s00383-008-2257-y
3. van den Hout L, Schaible T, Cohen-Overbeek TE, Hop W, Siemer J, van de Ven K, et al. Actual outcome in infants with congenital diaphragmatic hernia: the role of a standardized postnatal treatment protocol. *Fetal Diagn Ther.* (2011) 29:55–63. doi: 10.1159/000322694
4. Chen C, Jeruss S, Chapman JS, Terrin N, Tighiouart H, Glassman E, et al. Long-term functional impact of congenital diaphragmatic hernia repair on children. *J Pediatr Surg.* (2007) 42:657–65. doi: 10.1016/j.jpedsurg.2006.12.013
5. Catena F, Di Saverio S, Kelly MD, Biffl WL, Ansaloni L, Mandalà V, et al. Bologna guidelines for diagnosis and management of adhesive small bowel obstruction (ASBO): 2010 evidence-based guidelines of the world society of emergency surgery. *World J Emerg Surg.* (2011) 6:5. doi: 10.1186/1749-7922-6-5
6. Parker MC, Wilson MS, Menzies D, Sunderland G, Clark DN, Knight AD, et al. The SCAR-3 study: 5-year adhesion-related readmission risk following lower abdominal surgical procedures. *Colorectal Dis.* (2005) 7:551–8. doi: 10.1111/j.1463-1318.2005.00857.x
7. Hellebrekers BW, Kooistra T. Pathogenesis of postoperative adhesion formation. *Br J Surg.* (2011) 98:1503–16. doi: 10.1002/bjs.7657
8. Schultz C, Temming P, Bucsky P, Göpel W, Strunk T, Härtel C. Immature anti-inflammatory response in neonates. *Clin Exp Immunol.* (2004) 135:130–6. doi: 10.1111/j.1365-2249.2004.02313.x
9. Kobayashi H, Yamataka A, Okazaki T, Lane GJ, Puri P, Miyano T. Increased levels of circulating adhesion molecules in neonates with congenital diaphragmatic hernia complicated by persistent pulmonary hypertension. *Pediatr Surg Int.* (2004) 20:19–23. doi: 10.1007/s00383-003-1072-8
10. Menzies D, Ellis H. Intestinal obstruction from adhesions–how big is the problem? *Ann R Coll Surg Engl.* (1990) 72:60–3.
11. Ten Broek RPG, Krielen P, Di Saverio S, Coccolini F, Biffl WL, Ansaloni L, et al. Bologna guidelines for diagnosis and management of adhesive small bowel obstruction (ASBO): 2017 update of the evidence-based guidelines from the world society of emergency surgery ASBO working group. *World J Emerg Surg.* (2018) 13:24. doi: 10.1186/s13017-018-0185-2
12. Di Saverio S, Coccolini F, Galati M, Smerieri N, Biffl WL, Ansaloni L, et al. Bologna guidelines for diagnosis and management of adhesive small bowel obstruction (ASBO): 2013 update of the evidence-based guidelines from the world society of emergency surgery ASBO working group. *World J Emerg Surg.* (2013) 8:42. doi: 10.1186/1749-7922-8-42
13. Tabibian N, Swehli E, Boyd A, Umbreen A, Tabibian JH. Abdominal adhesions: a practical review of an often overlooked entity. *Ann Med Surg (Lond).* (2017) 15:9–13. doi: 10.1016/j.amsu.2017.01.021
14. Burgos CM, Modee A, Ost E, Frenckner B. Addressing the causes of late mortality in infants with congenital diaphragmatic hernia. *J Pediatr Surg.* (2017) 52:526–9. doi: 10.1016/j.jpedsurg.2016.08.028
15. Choudhry MS, Grant HW. Small bowel obstruction due to adhesions following neonatal laparotomy. *Pediatr Surg Int.* (2006) 22:729–32. doi: 10.1007/s00383-006-1719-3
16. Lakshminarayanan B, Hughes-Thomas AO, Grant HW. Epidemiology of adhesions in infants and children following open surgery. *Semin Pediatr Surg.* (2014) 23:344–8. doi: 10.1053/j.sempedsurg.2014.06.005
17. Lautz TB, Raval MV, Reynolds M, Barsness KA. Adhesive small bowel obstruction in children and adolescents: operative utilization and factors associated with bowel loss. *J Am Coll Surg.* (2011) 212:855–61. doi: 10.1016/j.jamcollsurg.2011.01.061
18. van Eijck FC, Wijnen RM, van Goor H. The incidence and morbidity of adhesions after treatment of neonates with gastroschisis and omphalocele: a 30-year review. *J Pediatr Surg.* (2008) 43:479–83. doi: 10.1016/j.jpedsurg.2007.10.027
19. Festen C. Postoperative small bowel obstruction in infants and children. *Ann Surg.* (1982) 196:580–3. doi: 10.1097/00000658-198211000-00012
20. Wilkins BM, Spitz L. Incidence of postoperative adhesion obstruction following neonatal laparotomy. *Br J Surg.* (1986) 73:762–4. doi: 10.1002/bjs.1800730929
21. Grant HW, Parker MC, Wilson MS, Menzies D, Sunderland G, Thompson JN, et al. Population-based analysis of the risk of adhesion-related readmissions after abdominal surgery in children. *J Pediatr Surg.* (2006) 41:1453–6. doi: 10.1016/j.jpedsurg.2006.04.023
22. Young JY, Kim DS, Muratore CS, Kurkchubasche AG, Tracy TF, Jr., Luks FI. High incidence of postoperative bowel obstruction in newborns and infants. *J Pediatr Surg.* (2007) 42:962–5. doi: 10.1016/j.jpedsurg.2007.01.030
23. Yokota K, Uchida H, Kaneko K, Ono Y, Murase N, Makita S, et al. Surgical complications, especially gastroesophageal reflux disease, intestinal adhesion obstruction, and diaphragmatic hernia recurrence, are major sequelae in survivors of congenital diaphragmatic hernia. *Pediatr Surg Int.* (2014) 30:895–9. doi: 10.1007/s00383-014-3575-x
24. Janssen S, Heiwegen K, van Rooij IA, Scharbatke H, Roukema J, de Blaauw I, et al. Factors related to long-term surgical morbidity in congenital diaphragmatic hernia survivors. *J Pediatr Surg.* (2018) 53:508–12. doi: 10.1016/j.jpedsurg.2017.05.032
25. Heiwegen K, de Blaauw I, van Ling J, Botden S. Malrotation in congenital diaphragmatic hernia: is it really a problem? *Eur J Pediatr Surg.* (2019). doi: 10.1055/s-0039-1692165
26. Lampl B, Levin TL, Berdon WE, Cowles RA. Malrotation and midgut volvulus: a historical review and current controversies in diagnosis and management. *Pediatr Radiol.* (2009) 39:359–66. doi: 10.1007/s00247-009-1168-y
27. Ward EP, Wang A, Thangarajah H, Lazar D, Bickler S, Fairbanks T, et al. Preemptive Ladd Procedure in congenital diaphragmatic hernia and Abdominal Wall defects does not reduce the risk of future volvulus. *J Pediatr Surg.* (2017) 52:1956–61. doi: 10.1016/j.jpedsurg.2017.08.067
28. Reiss I, Schaible T, van den Hout L, Capolupo I, Allegaert K, van Heijst A, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH EURO Consortium consensus. *Neonatology.* (2010) 98:354–64. doi: 10.1159/000320622
29. Snoek KG, Reiss IK, Greenough A, Capolupo I, Urlesberger B, Wessel L, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH EURO consortium consensus - 2015 update. *Neonatology.* (2016) 110:66–74. doi: 10.1159/000444210
30. Loff S, Wirth H, Jester I, Hosie S, Wollmann C, Schaible T, et al. Implantation of a cone-shaped double-fixed patch increases abdominal space and prevents recurrence of large defects in congenital diaphragmatic hernia. *J Pediatr Surg.* (2005) 40:1701–5. doi: 10.1016/j.jpedsurg.2005.07.007
31. Garner JS. CDC guideline for prevention of surgical wound infections, 1985. Supersedes guideline for prevention of surgical wound infections published in 1982. (Originally published in November 1985). *Revised Infect Control.* (1986) 7:193–200. doi: 10.1017/S0195941700064080
32. Heiwegen K, de Blaauw I, Botden S, A. systematic review and meta-analysis of surgical morbidity of primary versus patch repaired congenital diaphragmatic hernia patients. *Sci Rep.* (2021) 11:12661. doi: 10.1038/s41598-021-91908-7
33. Jancelewicz T, Vu LT, Keller RL, Bratton B, Lee H, Farmer D, et al. Long-term surgical outcomes in congenital diaphragmatic hernia: observations from a single institution. *J Pediatr Surg.* (2010) 45:155–60. doi: 10.1016/j.jpedsurg.2009.10.028
34. Gutt CN, Oniu T, Schemmer P, Mehrabi A, Büchler MW. Fewer adhesions induced by laparoscopic surgery? *Surg Endosc.* (2004) 18:898–906. doi: 10.1007/s00464-003-9233-3
35. Attard JA, MacLean AR. Adhesive small bowel obstruction: epidemiology, biology and prevention. *Can J Surg.* (2007) 50:291–300.
36. Brinkmann V, Zychlinsky A. Neutrophil extracellular traps: is immunity the second function of chromatin? *J Cell Biol.* (2012) 198:773–83. doi: 10.1083/jcb.201203170
37. Korkmaz HI, Ulrich MMW, Vogels S, de Wit T, van Zuijlen PPM, Krijnen PAJ, et al. Neutrophil extracellular traps coincide with a pro-coagulant status of microcirculatory endothelium in burn wounds. *Wound Repair Regen.* (2017) 25:609–17. doi: 10.1111/wrr.12560
38. Coccolini F, Ansaloni L, Manfredi R, Campanati L, Poiasina E, Bertoli P, et al. Peritoneal adhesion index (PAI): proposal of a score for the

"ignored iceberg" of medicine and surgery. *World J Emerg Surg.* (2013) 8:6. doi: 10.1186/1749-7922-8-6
39. Fugazzola P, Coccolini F, Nita GE, Montori G, Corbella D, Adesunkanmi ARK, et al. Validation of peritoneal adhesion index as a standardized classification to universalize peritoneal adhesions definition. *J Peritoneum (and other serosal surfaces).* (2017). doi: 10.4081/joper.2017.61
40. Sisodia V, Sahu SK, Kumar S. Clinical profile of patients with postoperative adhesive intestinal obstruction and its association with intraoperative peritoneal adhesion index. *Chirurgia (Bucur).* (2016) 111:251–8.
41. Choi ET, Lim SB, Lee JL, Kim CW, Kim YI, Yoon YS, et al. Effects of anchoring sutures at diverting ileostomy after rectal cancer surgery on peritoneal adhesion at following ileostomy reversal. *Ann Surg Treat Res.* (2021) 101:214–20. doi: 10.4174/astr.2021.101.4.214
42. Ruiz-Esparza GU, Wang X, Zhang X, Jimenez-Vazquez S, Diaz-Gomez L, Lavoie AM, et al. Nanoengineered shear-thinning hydrogel barrier for preventing postoperative abdominal adhesions. *Nanomicro Lett.* (2021) 13:212. doi: 10.1007/s40820-021-00712-5
43. van der Wal JB, Iordens GI, Vrijland WW, van Veen RN, Lange J, Jeekel J. Adhesion prevention during laparotomy: long-term follow-up of a randomized clinical trial. *Ann Surg.* (2011) 253:1118–21. doi: 10.1097/SLA.0b013e318217e99c
44. Heuer A, Stiel C, Elrod J, Konigs I, Vincent D, Schlegel P, et al. Therapeutic targeting of neutrophil extracellular traps improves primary and secondary intention wound healing in mice. *Front Immunol.* (2021) 12:614347. doi: 10.3389/fimmu.2021.614347
45. Putnam LR, Tsao K, Lally KP, Blakely ML, Jancelewicz T, Lally PA, et al. Minimally invasive vs open congenital diaphragmatic hernia repair: is there a superior approach? *J Am Coll Surg.* (2017) 224:416–22. doi: 10.1016/j.jamcollsurg.2016.12.050
46. Jancelewicz T, Chiang M, Oliveira C, Chiu PP. Late surgical outcomes among congenital diaphragmatic hernia (CDH) patients: why long-term follow-up with surgeons is recommended. *J Pediatr Surg.* (2013) 48:935–41. doi: 10.1016/j.jpedsurg.2013.02.005
47. Barroso C, Correia-Pinto J. Perioperative complications of congenital diaphragmatic hernia repair. *Eur J Pediatr Surg.* (2018) 28:141–7. doi: 10.1055/s-0038-1632374
48. Davenport M, Rothenberg SS, Crabbe DC, Wulkan ML. The great debate: open or thoracoscopic repair for oesophageal atresia or diaphragmatic hernia. *J Pediatr Surg.* (2015) 50:240–6. doi: 10.1016/j.jpedsurg.2014.11.008
49. Laituri CA, Garey CL, Valusek PA, Fike FB, Kaye AJ, Ostlie DJ, et al. Outcome of congenital diaphragmatic hernia repair depending on patch type. *Eur J Pediatr Surg.* (2010) 20:363–5. doi: 10.1055/s-0030-1261939
50. St Peter SD, Valusek PA, Tsao K, Holcomb GW. 3rd, Ostlie DJ, Snyder CL. Abdominal complications related to type of repair for congenital diaphragmatic hernia. *J Surg Res.* (2007) 140:234–6. doi: 10.1016/j.jss.2007.03.018
51. Grethel EJ, Cortes RA, Wagner AJ, Clifton MS, Lee H, Farmer DL, et al. Prosthetic patches for congenital diaphragmatic hernia repair: Surgisis vs Gore-Tex. *J Pediatr Surg.* (2006) 41:29–33. doi: 10.1016/j.jpedsurg.2005.10.005
52. Koehler RH, Begos D, Berger D, Carey S, LeBlanc K, Park A, et al. Minimal adhesions to ePTFE mesh after laparoscopic ventral incisional hernia repair: reoperative findings in 65 cases. *JSLS.* (2003) 7:335–40.
53. Montz FJ, Monk BJ, Lacy SM. The gore-tex surgical membrane: effectiveness as a barrier to inhibit postradical pelvic surgery adhesions in a porcine model. *Gynecol Oncol.* (1992) 45:290–3. doi: 10.1016/0090-8258(92)90306-4
54. Zahn KB, Schaible T, Rafat N, Weis M, Weiss C, Wessel L. Longitudinal follow-up with radiologic screening for recurrence and secondary hiatal hernia in neonates with open repair of congenital diaphragmatic hernia—a large prospective, observational cohort study at one referral center. *Front Pediatr.* (2021) 9. doi: 10.3389/fped.2021.796478
55. Molinaro F, Kaselas C, Lacreuse I, Moog R, Becmeur F. Postoperative intestinal obstruction after laparoscopic versus open surgery in the pediatric population: A 15-year review. *Eur J Pediatr Surg.* (2009) 19:160–2 doi: 10.1055/s-0029-1202858
56. Koivusalo A, Karila K, Pakarinen M. Late abdominal reoperations after surgery for necrotizing enterocolitis and spontaneous intestinal perforation. *Eur J Pediatr Surg.* (2021). doi: 10.1055/s-0040-1722616
57. Davila RM, Crouch EC. Role of mesothelial and submesothelial stromal cells in matrix remodeling following pleural injury. *Am J Pathol.* (1993) 142:547–55.
58. Tutor JD. Chylothorax in infants and children. *Pediatrics.* (2014) 133:722–33. doi: 10.1542/peds.2013-2072
59. Fevang BT, Fevang J, Lie SA, Soreide O, Svanes K, Viste A. Long-term prognosis after operation for adhesive small bowel obstruction. *Ann Surg.* (2004) 240:193–201. doi: 10.1097/01.sla.0000132988.50122.de
60. Williams SB, Greenspon J, Young HA, Orkin BA. Small bowel obstruction: conservative vs. surgical management. *Dis Colon Rectum.* (2005) 48:1140–6. doi: 10.1007/s10350-004-0882-7
61. Lautz TB, Barsness KA. Adhesive small bowel obstruction–acute management and treatment in children. *Semin Pediatr Surg.* (2014) 23:349–52. doi: 10.1053/j.sempedsurg.2014.06.006

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REVIEW
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Use of Prostaglandin E1 in the Management of Congenital Diaphragmatic Hernia–A Review

Srirupa Hari Gopal[1]*, *Neil Patel*[2] and *Caraciolo J. Fernandes*[1]

[1] Section of Neonatology, Department of Pediatrics, Baylor College of Medicine, Houston, TX, United States, [2] Department of Neonatology, Royal Hospital for Children, Glasgow, United Kingdom

Congenital diaphragmatic hernia (CDH) is a rare congenital anomaly, whose presentation is complicated by pulmonary hypertension (PH), pulmonary hypoplasia, and myocardial dysfunction, each of which have significant impact on short-term clinical management and long-term outcomes. Despite many advances in therapy and surgical technique, optimal CDH management remains a topic of debate, due to the variable presentation, complex pathophysiology, and continued impact on morbidity and mortality. One of the more recent management strategies is the use of prostaglandin E1 (PGE1) infusion in the management of PH associated with CDH. PGE1 is widely used in the NICU in critical congenital cardiac disease to maintain ductal patency and facilitate pulmonary and systemic blood flow. In a related paradigm, PGE1 infusion has been used in situations of supra-systemic right ventricular pressures, including CDH, with the therapeutic intent to maintain ductal patency as a "pressure relief valve" to reduce the effective afterload on the right ventricle (RV), optimize cardiac function and support pulmonary and systemic blood flow. This paper reviews the current evidence for use of PGE1 in the CDH population and the opportunities for future investigations.

Keywords: Congenital Diaphragmatic Hernia (CDH), pulmonary hypertension, prostagladin E1, Patent Ductus Arteriosus (PDA), ventricular dysfunction

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***Correspondence:**
Srirupa Hari Gopal
srirupa.harigopal@bcm.edu

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INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a rare anomaly, characterized by a defect in the diaphragm causing abdominal contents to protrude into the thoracic cavity. The incidence of CDH is 1 in 2,500 to 1 in 3,500 live births (1). It occurs 70%−75% of the time in the posterolateral aspect of the diaphragm, with over 85% occurring on the left side (2). CDH can also be associated with congenital heart defects (25%−40%), urogenital anomalies (18%), musculoskeletal anomalies (16%) and central nervous system anomalies (10%) (3, 4). Despite medical and surgical advances, CDH continues to have high mortality and morbidity rates (5). Pulmonary hypoplasia and pulmonary hypertension (PH) are hallmarks of CDH presentation, resulting from both pulmonary vasculature and respiratory maldevelopment, the severity of which determine outcomes. In addition to these factors, there is also an increasing appreciation of early postnatal cardiac dysfunction as a determinant of outcome in CDH and use of agents to ameliorate cardiac dysfunction (6, 7).

Prostaglandin E1 (PGE1), a prostaglandin analog administered by intravenous infusion, is typically used to maintain ductal patency in newborn infants in the setting of suspected duct-dependent congenital heart disease. In this setting, PGE1 infusion is used to ensure adequate systemic or pulmonary blood flow or mixing until definitive surgical repair is accomplished. In the setting of pulmonary hypertensive disease, including CDH, PGE1 may have other benefits, the most important being reduction of afterload of a failing right ventricle (RV).

In this review, we will discuss the pathophysiology of CDH, the theoretical benefits of PGE1 therapy in the management of CDH, critically review existing evidence of its use, and identify key questions for future areas of research.

PATHOPHYSIOLOGY OF CDH

Congenital diaphragmatic hernia is associated with pulmonary hypoplasia of varying extent typically affecting both the ipsilateral and contralateral lungs. This abnormal pathophysiology has been hypothesized to begin at ∼8th to 10th-week of gestation, after failure of the normal physiological closure of the diaphragm and the establishment of the separation between abdominal and thoracic organs (8). A 2-hit hypothesis has been proposed to explain this spectrum of pulmonary hypoplasia. Following the initial "hit", possibly genetic or environmental, during the early stages of organ development, bilateral lung hypoplasia occurs. This is followed by the second "hit" - compression of the ipsilateral lung by the hernia itself (9). Accompanying these changes in lung architecture are changes in the pulmonary vasculature, specifically early maturation, underdevelopment and increased muscularization of the pulmonary arterial vessels leading to altered vessel tone and reduced vessel caliber (10). Molecular pathways that are implicated in pulmonary vascular remodeling in CDH, which have been studied in humans and nitrofen rat models (11), include the retinol pathway (12), vascular endothelial growth factor (VEGF) (13), endothelin (14), Bone Morphogenic Protein (BMP) and Apelin (15). Alternations in these pathways may affect endothelial cell function, molecular signaling to the pulmonary arterial smooth muscle cells contributing to pulmonary arterial smooth muscle cell proliferation, and the characteristically hypertrophic pulmonary arterioles found in CDH-associated PH (CDH-PH) (6, 16).

The pulmonary alveolar and vascular maldevelopment results in increased pulmonary vascular resistance and associated PH. Studies have shown that over 70% of CDH infants exhibit CDH-PH (17), which is independently associated with increased mortality risk, oxygen support at 30 days, and utilization of extracorporeal life support (ECLS) (18). PH manifests as hypoxia due to right-to-left shunting across the atria, patent ductus arteriosus and any ventricular septal defect, if present, as well as increased afterload on the RV. In response to the increased afterload, the RV exhibits an initial adaptive dilatory response that may be followed by maladaptive hypertrophy and subsequent failure, which may be exacerbated by a restrictive ductus arteriosus. This RV failure may in turn result in impairment of diastolic filling of the left ventricle (LV) and reduced systemic blood flow. Myocardial ischemia of the RV plays an important role in the pathophysiology of cardiac failure in PH; specifically, through compromised right coronary blood flow. In PH, the right coronary perfusion gradient may be reduced due to the sustained increase in RV pressures and decrease in the aortic pressures (due to reduced LV preload and cardiac output) (19). Decreased RV coronary perfusion in the context of increasing myocardial oxygen consumption may predispose the RV to ischemia and dysfunction (20).

In addition, CDH has been described to be associated with both structural and functional left ventricular abnormalities (7, 21, 22). Fetal LV hypoplasia is well-described and possibly occurs due to mechanical compression, reduced fetal LV blood flow from reduced pulmonary venous return and altered streaming of venous return due to mediastinal shift (23–25). In a LV that may already be relatively hypoplastic due to the aforementioned reasons, the increase in afterload during the transition at birth, combined with the interdependent impacts of RV dilatation and dysfunction, can lead to significant LV dysfunction with adverse cardiopulmonary and hemodynamic outcomes (26). In a recent study, Patel et al. reported that early LV systolic function correlated with prenatal and postnatal markers of clinical disease severity (27). This observation underscores the importance of appropriate management of early PH in order to prevent biventricular dysfunction and associated impairment of systemic blood flow and oxygen delivery.

The combination and spectrum of pulmonary hypoplasia, pulmonary hypertension, and ventricular dysfunction makes CDH a unique clinical management challenge. Historically, PH therapeutic strategies in CDH have focused mainly on pulmonary vasodilation. The main therapeutic targets are cytokine pathways regulating pulmonary artery smooth muscle tone, specifically such the nitric oxide (NO) pathway, prostacyclin pathway and endothelin pathways (28).

Inhaled nitric oxide (iNO), a potent pulmonary vasodilator, acts by stimulating guanylyl cyclase in the vascular smooth muscle cells to produce cyclic guanosine monophosphate (cGMP). Elevated intracellular concentrations of cGMP activate cGMP-dependent protein kinases and lower cytosolic calcium concentrations, which in turn promote vascular smooth muscle cell relaxation (29). Phosphodiesterases (PDEs) are a large family of enzymes that hydrolyze cyclic nucleotides (cGMP and cAMP). Inhibition of PDEs leads to vasodilator effects. PDE5 (a cGMP-specific PDE), PDE3 and 4 (which hydrolyze cAMP) are expressed in the lung (28). Sildenafil, a PDE5 inhibitor that acts *via* the NO pathway, has been widely used in the management of CDH-PH (30). Milrinone, a PDE3 inhibitor, has also been studied in the CDH population (28, 31). Another important pulmonary vasodilator is prostacyclin; agents targeting this pathway include epoprostenol and inhaled iloprost (28, 32, 33). Inhibition of PDE3 causes lower pulmonary arterial pressures by acting *via* the PGI2 pathway (28). Endothelin (ET)-1 is a potent vasoconstrictor, and, hence, a target for modulating pulmonary vascular resistance. In a randomized control trial comparing the use of Bosentan, a drug which acts on the ETA

and ETB receptors, with placebo as treatment for neonates with persistent pulmonary hypertension (PPHN), Mohamed et al. reported that Bosentan was superior to placebo for the treatment of PPHN (34).

THE RV IN PH DISEASE: ANIMAL MODELS

Among studies in adults with PH, the major cause of mortality of patients with PH was RV failure (35). Experimental animal models to investigate the effect of pressure overload on the RV include the monocrotaline (MCT) and chronic hypoxic mouse models (36) and the pulmonary artery banding (PAB) mouse model (37) which was developed to study the RV-specific effects independent of the pulmonary circulation. RV failure molecular mechanisms involve abnormal metabolism, impaired angiogenesis, mitochondrial dysfunction and increased oxidative stress (38–40). Further, the "sick lung circulation" hypothesis postulates that altered lung vascular cells from the "sick lung", such as those containing cell fragments, free DNA and microRNA, can be cytotoxic to the RV and can re-program endothelial cell genes, thus contributing to the RV failure (40).

Several important concepts regarding ventricular response to elevated pulmonary pressures investigated in animal models can be translated to clinical medicine. Firstly, as demonstrated by Urashima et al. (41), the RV and LV do not respond identically to pressure overload; thus, treatment strategies that focus individually and specifically to each ventricle are important. Additionally, it has been shown that acute RV pressure overload impairs LV function by altering septal strain and apical rotation (42). The RV's molecular adaptation varies based on the degree of pressure overload as well as the type of pressure overload (proximal type as seen in the PAB model vs peripheral type seen in PH models vs combined pressure and volume overload as in the presence of a shunt) (43, 44). Severe PH can result not only in systolic, but also diastolic dysfunction (45). Hemodynamic measurements of the RV in response to PH have been shown to correlate and predict biomechanical changes in the myocardium (46). Pressure overload on the RV significantly alters the pressure-volume relationship, leading to greater end-diastolic pressures, and concurrently increasing the longitudinal elastic modulus [Elastic modulus (E) or the amount of force required to deform a tissue] in the PAB rat model (46).

Existing studies of RV function in CDH, though limited, indicate similar morphological and functional changes. Echocardiographic studies of early RV function have demonstrated RV systolic and diastolic dysfunction, and evidence of interdependent impairment of LV function (47, 48). Furthermore, early RV dysfunction pre- and post CDH repair have been shown to be associated with adverse outcomes, including increased mortality, ECLS use and length of hospitalization in survivors, in single center cohorts as well as large registry-based analyses (49–51).

An important conclusion that can be drawn from these animal model studies and clinical studies in CDH is the importance of unloading the RV in the setting of elevated pulmonary pressures, and tailoring PH therapy to target both biomechanical and hemodynamic function with the aim of optimizing RV function and improving outcomes.

ROLE OF PGE1 IN CDH

Prostaglandin E1 is a potent dilator of the ductus arteriosus in human neonates (52). The first studies ex-vivo in fetal lambs in 1973 by Coceani and Olley (53) led to clinical trials (54, 55) and approval for use by Food and Drug Administration (FDA) in 1981 (56).

The therapeutic benefits that PGE 1 offers in the setting of elevated pulmonary vascular resistance in CDH are theoretically three-fold. This has been summarized in **Figure 1**.

1) *By acting as pressure "blow-off" valve, reducing the effective afterload on the pressure loaded RV, alleviating RV dilatation and myocardial dysfunction*. LV function in turn may also improve by mechanisms of ventricular interdependence. A similar strategy of having a "pop off" conduit in supra-systemic pulmonary pressures has been demonstrated by the use of the Pott's shunt (anastomosis between left pulmonary artery to descending aorta) in pediatric hypertension and in patients with Eisenmenger syndrome (57). Evidence from pediatric patients with pulmonary hypertension have shown that a Pott's shunt improves RV-systolic function and RV-PA coupling, resulting in overall improved functional status and transplant-free survival (58).
2) *By augmenting systemic blood flow in the setting of LV failure, by facilitating right-to-left shunting via the ductus arteriosus.* The evidence of the benefits of using PGE1 to augment systemic blood flow is best noted in single ventricle pathologies such as hypoplastic left heart syndrome, where there exists an uncertain balance among systemic, pulmonary and coronary blood flows, with the systemic and pulmonary circulations in parallel rather than in series. The use of PGE1 in this situation ensures systemic blood flow to the vital organs, and also balances the systemic and pulmonary cardiac output (59).
3) *By its direct pulmonary vasodilating action in pulmonary artery smooth muscle*. PGE1 increases intracellular cyclic AMP leading to decreased pulmonary vascular resistance (60), reducing RV afterload and potentially improving coronary perfusion to the RV (20). The pulmonary vasodilator benefits of PGE1 in primary pediatric pulmonary hypertension (61) and in neonatal PPHN have been demonstrated previously (62, 63).

Animal studies support these potential benefits. Sakuma et al. (64), demonstrated in a monocrotaline rat PH model that PGE1 administration significantly reduced the production of cytokines IL-1, IL-6 and TNF, previously implicated in pulmonary hypertension. In another study by Ono et al. (65), PGE1 had a dose-dependent suppression of RV hypertrophy and pulmonary hypertension in a MCT rat model.

Though the potential benefits of PGE1 use in the cardiopulmonary physiology of CDH appear compelling, there are potential adverse effects. In the short-term, PGE1 may

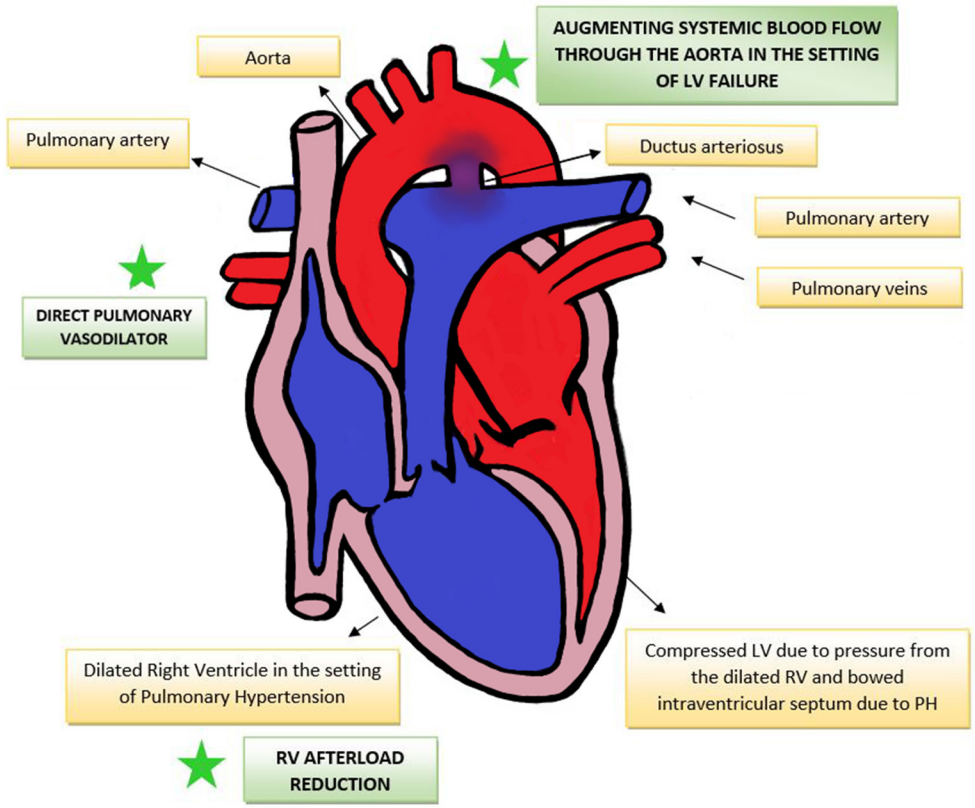

FIGURE 1 | Effect of PGE1 in PH-RV afterload reduction, direct pulmonary vasodilation and augmenting systemic blood flow. RV, right ventricle; LV, left ventricle; PH, pulmonary hypertension.

induce apnea, peripheral vasodilation, fever and hypotension (66). With long term use (>5 days), cortical hyperostosis, brown fat necrosis, gastric outlet obstruction and intimal mucosal damage have been reported (66). Worsening hypoxia due to right-to left ductal shunting should also be considered (67).

REVIEW OF CLINICAL STUDIES

To date, the investigation of PGE1 use in CDH has been limited to case reports and retrospective chart reviews. We performed a review of literature using the electronic bibliographic databases PubMed and Embase, and of ongoing trials in www.clinicaltrials.gov. Additionally, we also used PubMed's related citations feature to identify relevant studies. We included chart reviews, case control studies, case series and case reports. Once a list of studies was obtained, we analyzed the studies for methodology and outcomes measures as described below.

A summary of these studies is provided in **Table 1**.

Methodology and Indications of PGE1 Use

All but one of the studies of PGE1 use in CDH-PH have been retrospective chart reviews, comparing patients who received PGE1 with those who did not (26, 68–70), one retrospective study compared the combined therapy of PGE+iNO with those only receiving iNO (23). Three studies reported initiating PGE1 based only on echocardiographic parameters. Inamura et al. (68, 69) reported initiating PGE1 infusion when the duration of right-to-left shunting *via* the DA was longer than that of left-to-right shunting, whereas Shiyanagi et al. (23) reported using PGE1 for PH based on echocardiographic signs: dominant right-to-left shunt through a PDA, decrease in pulmonary arterial blood flow on the affected side, and tricuspid regurgitation velocity (TRV) more than 2.5 m/s. Two of the studies reported using PGE1 based on a specific criterion; Lawrence et al. delineated specific indications for PGE1 initiation based on institutional CDH guidelines, which included (1) echocardiographic findings of PH with a restrictive PDA, (2) persistent metabolic acidosis (pH < 7.25 with base deficit or elevation of lactate) without left heart dysfunction on echocardiography, or (3) persistent post-ductal arterial oxygen content <30 mmHg (70). In the study by Le Duc et al. (26), PGE1 was initiated when the maximal right-to-left blood flow velocities were >1.5m/s in CDH infants with acute worsening of the cardiorespiratory status.

As noted, most of the cited studies specified a right-to-left ductal shunting pattern as an indication for initiation of PGE1. One study mentioned a "restrictive PDA" as a criterion, but no specific duct size measurement was reported (70). None of the studies report echocardiographic evidence of abnormal

TABLE 1 | Summary of clinical studies on PGE1 use in CDH.

Author	Year of publication & country	Study design	n	Indications for PGE1	Age at use	Dosing of PGE	Duration of therapy (Days)	Echocardiographic assessment	Outcome measures	Results/outcome of the study
Inamura et al. (68)	2005 (Japan)	Retrospective review	Total-19; PGE (+) 9	Duration of the R-L shunt through the DA was longer than that of L–R shunt	–	3–5 ng/kg/min	-	1. LV diastolic diameter index [LVDI] 2. Total pulmonary artery index [TPAI] 3. LV-Tei index Measured on DOL 0 & 2	Echocardiographic markers of LV dysfunction	1. LVDI and TPAI of day 0 in PG (+) were significantly smaller 2. LV Tei index on postnatal day 0 in PG (+) was significantly higher
Shiyanagi et al. (23)	2008 (Japan)	Retrospective review	PG+iNO-19, iNO-30	Echo signs of PH	–	0.05–0.20 μg/kg/min	–	1. Dominant R-L shunt through a PDA 2. Decrease in pulmonary arterial blood flow on the affected side. 3. Tricuspid regurgitation velocity (TRV) > 2.5 m/s.	1. Survival rate 2. Length of hospital stay 3. Timing of surgical repair 4. Timing of spontaneous close of DA	1. No significant difference between survival rates between the groups 2. Hospital stay was significantly shorter in the iNO group 3. Earlier surgery in iNO group 4. Spontaneous closure of PDA was early in iNO group
Inamura et al. (69)	2014 (Japan)	Retrospective review	Total-61 PGE (+)39 PGE (–) 22	Duration of the R-L shunt through the DA was longer than that of L–R shunt	–	3–5 ng/kg/min	–	1. The LV end-diastolic diameter, corrected for body surface area (LVDD/BSA) 2. Ejection fraction (EF), 3. Tei index-LV	Echocardiographic markers of LV dysfunction	1. Improved LV function shown by significant increase in LVDD and LV-Tei index

(Continued)

TABLE 1 | Continued

Author	Year of publication & country	Study design	n	Indications for PGE1	Age at use	Dosing of PGE	Duration of therapy (Days)	Echocardiographic assessment	Outcome measures	Results/outcome of the study
Lawrence et al. (70)	2019 (USA)	Retrospective review	PGE (+)57	1) Echo findings of PH with a restrictive PDA 2) Persistent metabolic acidosis (pH< 7.25 with base deficit or lactate elevation) without LV dysfunction on echo, or 3) Post ductal arterial oxygen content <30 mmHg.	DOL 9 (IQR 2–13)	0.01–0.05 μg/kg/min	17±2	1. TR jet velocity 2. DA direction 3. Septal position	1. BNP levels 2. Echo markers of severe PH	1. BNP levels declined after 1.4 ± 0.2 days and again at 5.2 ± 0.6 days after treatment 2. Echo markers of severe PH improved significantly, after 6 ± 0.8 days of treatment
Le Duc et al. (26)	2020 (France)	Retrospective review	PGE (+)-18	Maximal R-L blood flow velocities are > 1.5m/s with acute worsening of the cardiorespiratory status	DOL 11 (IQR 5–16)	0.025–0.05 μg/kg/min	3	1. Maximal blood flow velocities and flow patterns through the DA 2. Mean PAP compared to the systolic blood pressure measured on echo and classified as supra-systemic when mean PAP is ≥ systemic blood pressure + 10 mmHg	1. Decrease in FiO_2 2. Ductal flow direction and velocities	1. Significant decrease in FiO_2 at hour 6 (median FiO_2 decreased from 80% to 34% to target preductal SpO_2 between 88% and 96%) 2. Significant decrease in maximal blood flow velocities in the DA

PGE1, Prostaglandin E1; R-L, Right to left; L–R, Left to Right; DA, Ductus Arteriosus; RV-right ventricle; LV, Left Ventricle; iNO, Inhaled Nitric Oxide; PDA, Patent Ductus Arteriosus; PH, Pulmonary Hypertension; PAP-Pulmonary Arterial Pressure; BNP, Brain Natriuretic Peptide; DOL, Day of Life.

RV size or function as a marker for PGE1 initiation, although clinical signs of RV/LV failure were used as criteria in two of the cited studies (26, 70). One study used plasma BNP before and after PGE1 initiation as a measure of PH and RV strain, and demonstrated decline in BNP measurements and improvement in echocardiographic measures PH (70). Plasma BNP peptides are secreted in response to wall stress by both the ventricles. However, a study by Koch et al. demonstrated the rapid decrease in plasma BNP levels during the first week of life, and the use of plasma BNP as a marker of clinical improvement may not necessarily reflect the effect of PGE1 use (71).

Outcome Measures to Assess Response to PGE1

Echocardiographic markers have been used to assess the effect of PGE1: two studies used LV size and function (measuring LV diastolic diameter, total pulmonary artery index (TPAI), left ventricular end-diastolic diameter and LV-Tei index (a composite measure of LV function based on systolic and diastolic time intervals) (68, 69). One study used echocardiographic markers of PH (estimated RV systolic pressure using tricuspid regurgitation jet velocity, direction of flow across a patent ductus arteriosus, and ventricular septal position) (70), and one study reported ductal flow direction and velocities (26).

In the studies by Inamura et al. (68, 69), the authors concluded that in instances of severe PH keeping the ductus open plays an important role in the circulatory management of these patients by improving LV function (as indicated by a higher LV Tei index in infants receiving PGE1). Lawrence et al. (70) observed that use of PGE1 significantly reduced B-Natriuretic peptide levels (BNP, a plasma biomarker of pulmonary hypertension and associated cardiac strain) and echocardiographic indices of PH, as assessed by tricuspid regurgitation jet velocity, ductus arteriosus direction, and ventricular septum position. Le Duc et al. (26) concluded that use of PGE1 in CDH decreased FiO_2 requirements (median FiO_2 decreased from 80% to 34% to target preductal SpO_2 between 88 and 96%), and improved circulatory function, thus preventing cardiorespiratory failure in this population. Echocardiographic markers for PH used in this study include ductal flow velocities and flow patterns and mean pulmonary arterial pressures in relation to systemic blood pressures and classified as suprasystemic when mean PAP > systemic blood pressure +10 mm Hg (26). An observational study conducted by Hofmann et al. (72) reported that use of PGE1 in addition to circulatory management with catecholamines in two of their patients with CDH-PH relieved and stabilized right ventricular function. Although these studies reported on common echocardiographic markers of PH and LV dysfunction parameters, it is notable that none of the cited studies assessed RV function.

Most of the studies report improvement in cardiopulmonary outcomes with the use of PGE1 in CDH. However, a retrospective study by Shiyanagi et al. (23) demonstrated no significant clinical effects with the use of PGE1 combined with iNO, and concluded that use of iNO alone would simplify the management of PH due to CDH. The study reported no significant difference in survival to discharge, however, a shorter duration of hospitalization and earlier dates of repair were observed for those receiving iNO alone (23). Interestingly, this was also the only study to report PDA diameter, timing of spontaneous PDA closure and other long-term outcomes such as length of stay and survival to discharge (23).

In terms of adverse effects of PGE1, Shiyanagi et al. (23) reported lower systemic BP in the group that received PGE1 in comparison to those that did not. Lawrence et al. (70) reported seven patients with side-effects due to PGE1 which included pulmonary overcirculation due to L–R shunting (2%), cortical proliferation of their long bones (5%), temperature elevation (1.8%) and GI bleed (1.8%). However, none of the studies reported any life-threatening adverse effects or mortality attributed to the use of the medication.

In addition to the above studies, case reports describing improvement of cardio-respiratory function following use of PGE1 infusion are summarized in **Table 2** (73–76).

The above-described retrospective clinical studies and case reports indicate a potential cardio-respiratory benefit in using PGE1 in the managing PH in the CDH population. Why then is PGE1 not a routine component of the management of patients with CDH? (77, 78). Likely factors include uncertainty in identifying the appropriate subset of patients with CDH-PH, who might potentially benefit from this approach, and possibly the concerns relating to the short-term adverse effects and/or long-term adverse effects of having the ductus open. Possible ways to address these relevant concerns are two-fold:

1) Advocating for a more pronounced pathophysiology-based approach using serial echocardiograms.
2) Promoting further research on the use of PGE1 in this patient population to address these clinical concerns.

To our knowledge, there have not been any prospective studies investigating the effect of PGE1 on cardiorespiratory outcomes in CDH. Studies on significant and/or long-term outcomes, such as need for ECMO, duration of ventilation, length of stay, need for oxygen at discharge, need for additional PH medications and neurodevelopmental outcomes, are lacking. Use of PGE1 infusion either alone or in combination with other PH management strategies is a potential area for future research, which may further open the doors to other aspects of research such as the long-term effects of the presence of a ductus in patients with congenital diaphragmatic hernia.

FUTURE INVESTIGATIONS OF PGE1 INFUSION IN CDH

Ongoing areas of uncertainty about the use of PGE1 in CDH which require further investigation include:

1) Appropriate timing of the PGE1 infusion in CDH (e.g., earlier prophylactic administration vs. later after echocardiographic evidence of PH).
2) Dosing regimens of PGE1 for PH (e.g., fixed dosing or dose titration based on clinical and echocardiographic response). The studies described above have used variable dosing

TABLE 2 | Summary of case reports on PGE1 use in CDH.

Author	Year of publication & country	Patient description	Indications	Age at use (DOL)	Dosing of PGE	Duration of therapy	Echocardiographic assessment after PGE1 use and at discharge	Discharge (DOL)	PH therapy at discharge
Buss et al. (73)	2006 (Australia)	GA-41 weeks BW-3,094 g	DOL8 Echo- 1) Suprasystemic PAP 2) Severe TR with a pressure gradient in excess of 100 mm Hg and 3) Near complete closure of DA 4) Clinical signs of RV failure	8	10 ng/kg/min	20 days	**After PGE1**-Good RV function with only mild tricuspid valve regurgitation and reversion to bidirectional shunting across the PDA **At Discharge**- spontaneous closure of the duct and a fall of PAP to half systemic levels, mild tricuspid valve regurgitation. Good RV function	69	None
Filan et al. (74)	2006 (Australia)	GA-34 weeks BW-1,907 g	DOL 12- Echo- 1) Severe PH (based on TR jet) and right heart failure, 2) Systolic RV pressures equal to twice systemic, 3) Ventricular septum was bowing into LV cavity, 4) Closed DA	12	10 ng/kg/min	8 days	**After PGE1-**Ductal patency, reduction of RV pressures and improved RV function **At Discharge-**Good right ventricular function, a closed duct, and L–R interatrial shunting	54	
Divekar et al. (75)	2015 (USA)	GA- Full term BW-4,000 g	DOL1- Echo- 1) Restrictive PDA with R-L shunt, 2) Severe TR predicting supra-systemic PAP (TR 95 mm Hg, SBP 55/40 mm Hg), 3) Severe right ventricular (RV) dilation with septal bulge into the left ventricle (LV), and 4) Moderately reduced RV systolic function	1	0.05 mcg/kg/min	32 hours	**After PGE1**-Non-restrictive PDA with right to left shunting, improved RV systolic function, reduction in severity of TR, reduction in PAP from supra-systemic to systemic (TR 65 mm Hg, SBP 65/45 mm Hg), decreased septal shift (less LV compression) **At Discharge**- sub-systemic PAP	14	Sildenafil
Aljohani et al. (76)	2020 (USA)	GA-full term BW-3,280 g Prenatal markers- O/E TFLV 25%	DOL9-Echo- 1) RV pressure >2× systemic pressure based on TR jet 2) Septal motion, 3) Small PDA with R-L shunt, 4) Diminished RV function	9	-	41 days	**After PGE1-** Reduction in RV systolic pressures **At Discharge-**Normal septal position and RV size	78	Sildenafil Bosentan

GA, gestational age; BW, birth weight; DOL, day of life; TR, tricuspid regurgitation; PAP, pulmonary arterial pressure; PGE1, prostaglandin E1; R-L, right to left; L–R, Left to Right; DA, Ductus Arteriosus; RV-right ventricle; LV, Left Ventricle; iNO, Inhaled Nitric Oxide; PDA-Patent Ductus Arteriosus; PH-Pulmonary Hypertension; PAP-Pulmonary Arterial Pressure; O/E TFLV-Observed to Expected Total Fetal Lung Volume.

patterns for PGE1 use in CDH. Although this area may need to be further investigated, higher doses of PGE1 may have utility in situations of acute severe PH with RV failure with duct closure, analogous to those used in resuscitation of infants presenting with duct closure in critical congenital heart disease.
3) Potential side effects of its use in this population (both short- and long-term).
4) Duration of therapy (e.g., fixed or based on clinical and echocardiographic response).
5) Use of PGE1 in relation to ECLS.
6) Impact of concomitant use of other pulmonary vasodilators, such as iNO, Sildenafil and/or Bosentan.
7) Impact on LV/RV performance.
8) The specific subset of CDH that might best benefit from it use, which may require a pathophysiology-based, targeted approach to PH management in CDH.

In terms of "long-term" effects, areas of uncertainty ripe for further investigation include:

1) Impact on short- and long-term outcomes, including survival, need for ECLS, duration of ventilation, duration of hospital stay, need for oxygen at discharge, and neurodevelopmental outcome.
2) Timing and need for additional PH medications, such as Sildenafil and/or Bosentan at discharge.
3) Impact on intervention for ductal management due to the potential effect from L–R shunting.

With the evidence from a recent study that the severity of early postnatal PH has a significant impact on long-term outcome, an important question to be answered is the timing of the first echocardiogram in this population (18). This study stressed the importance of an early echocardiogram as a valuable prognostic tool that could potentially provide information that can impact the clinical course and management of PH.

Theoretically a well-designed clinical trial of PGE1 in CDH may help to address the current evidence gap. Ideally this would be a randomized double-blinded placebo trial of PGE1 in a priori risk-stratified subgroups of CDH patients with echocardiographic and clinical evidence of elevated PAP, biventricular dysfunction and a restrictive ductus and with outcome measures that include cardiopulmonary outcomes such as RV and LV performance, need for ECLS, effect of ventilatory needs, vasopressor needs, survival at discharge and mortality, managed using standardized management guidelines. Risk stratification should include prenatal imaging markers of severity, such as percent liver herniation (%LH), observed to expected Total Fetal Lung Volume (O/E TFLV), observed to expected Lung-Head Ratio (O/E LHR) (79), location of birth (in-born vs. out-born patients), etc.

Previous milestone trials in CDH include Ventilation in Infants with Congenital diaphragmatic hernia (VICI), the TOTAL trial of fetal tracheal occlusion, and the Neonatal Inhaled Nitric Oxide Study (NINOS) (80–82). Others are in progress include Congenital Diaphragmatic hernia Nitric Oxide vs. Sildenafil (CoDiNOS) trial and a randomized pilot trial of milrinone in congenital diaphragmatic hernia (31, 83). However, study recruitment for well-powered trials is a common challenge, which unfortunately can lead to smaller pilot studies without adequate power (84). Research in the CDH population is challenging due to small number of patients with isolated CDH and lack of evidence-based treatment strategies. Registry-based studies may be useful, but the wide variability in CDH management amongst institutions within the registry limit researchers' ability to draw meaningful conclusions or extrapolate the results to clinical practice (84). Investigating a therapeutic strategy of the use of PGE1 in patients with CDH, based on their clinical markers and echocardiographic indices of RV/LV dysfunction, could be thought of as a "pathophysiology-based approach" toward promoting precision medicine in this population. Such trials are sorely needed and will likely require multi-center collaboration to be completed in a timely fashion.

CONCLUSION

In conclusion, with continuing research to improve cardio-pulmonary and long-term outcomes in CDH, new management strategies are being proposed and studied. Supra-systemic RV pressures are associated with poor clinical outcomes in this population. There is a pathophysiological rationale for the use of PGE1 in CDH to maintain ductal patency and promote right-to-left shunting, thereby reducing effective RV afterload and supporting systemic blood flow. In addition, PGE1 may have direct pulmonary vasodilating actions. Although existing, single-center retrospective studies and case reports suggest benefit from the use of PGE1 in terms of reducing severity of PH and improving short-term cardiopulmonary stability, uncertainties remain around its optimal pragmatic clinical use in CDH, and current evidence from these studies may not strongly support clinical recommendations. Conducting pharmacological trials in neonates can be challenging due to physiological changes, variable pharmacokinetics in the early newborn period and the ethical considerations involved. However, a well-designed a-priori prospective study as outlined above should be considered to definitively understand the implications of the use of PGE1 in CDH and its impact on meaningful outcomes.

AUTHOR CONTRIBUTIONS

SH: conceptualization, design, methodology, and drafting and revising. NP: design, methodology, and reviewing and revising. CF: conceptualization, design, reviewing and revising, and supervision. All authors contributed to manuscript revision, read, approved the submitted version and agreed to be accountable for all aspects of the work.

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REFERENCES

1. McHoney M. Congenital diaphragmatic hernia. *Early Hum Dev.* (2014) 90:941–6. doi: 10.1016/j.earlhumdev.2014.09.013
2. Chandrasekharan PK, Rawat M, Madappa R, Rothstein DH, Lakshminrusimha S. Congenital diaphragmatic hernia–a review. *Matern Health Neonatol Perinatol.* (2017) 3:1–6. doi: 10.1186/s40748-017-0045-1
3. Scott DA, Gofin Y, Berry AM, Adams AD. Underlying genetic etiologies of congenital diaphragmatic hernia. *Prenat Diagn.* (2022) 42:373–86. doi: 10.1002/pd.6099
4. Stoll C, Alembik Y, Dott B, Roth MP. Associated malformations in patients with esophageal atresia. *Eur J Med Genet.* (2009) 52:287–90. doi: 10.1016/j.ejmg.2009.04.004
5. Brandt JB, Werther T, Groth E, Küng E, Golej J, Berger A. Risk factors for mortality in infants with congenital diaphragmatic hernia: a single center experience. *Wien Klin Wochenschr.* (2021) 133:674–9. doi: 10.1007/s00508-021-01843-w
6. Gupta VS, Harting MT. Congenital diaphragmatic hernia-associated pulmonary hypertension. *Semin Perinatol.* (2020) 44:151167. doi: 10.1053/j.semperi.2019.07.006
7. Kinsella JP, Steinhorn RH, Mullen MP, Hopper RK, Keller RL, Ivy DD, et al. The left ventricle in congenital diaphragmatic hernia: implications for the management of pulmonary hypertension. *J Pediatr.* (2018) 197:17–22. doi: 10.1016/j.jpeds.2018.02.040
8. Kluth D, Tenbrinck R, von Ekesparre M, Kangah R, Reich P, Brandsma A, et al. The natural history of congenital diaphragmatic hernia and pulmonary hypoplasia in the embryo. *J Pediatr Surg.* (1993) 28:456–63. doi: 10.1016/0022-3468(93)90248-J
9. Keijzer R, Liu J, Deimling J, Tibboel D, Post M. Dual-hit hypothesis explains pulmonary hypoplasia in the nitrofen model of congenital diaphragmatic hernia. *Am J Pathol.* (2000) 156:1299–306. doi: 10.1016/S0002-9440(10)65000-6
10. Sluiter I, van der Horst I, van der Voorn P. Boerema-de Munck A, Buscop-van Kempen M, de Krijger R, et al. Premature differentiation of vascular smooth muscle cells in human congenital diaphragmatic hernia. *Exp Mol Pathol.* (2013) 94:195–202. doi: 10.1016/j.yexmp.2012.09.010
11. Kluth D, Kangah R, Reich P, Tenbrinck R, Tibboel D, Lambrecht W. Nitrofen-induced diaphragmatic hernias in rats: an animal model. *J Pediatr Surg.* (1990) 25:850–4. doi: 10.1016/0022-3468(90)90190-K
12. Thébaud B, Tibboel D, Rambaud C, Mercier JC, Bourbon JR, Dinh-Xuan AT, et al. Vitamin A decreases the incidence and severity of nitrofen-induced congenital diaphragmatic hernia in rats. *Am J Physiol Lung Cell Mol Physiol.* (1999) 277:L423–9. doi: 10.1152/ajplung.1999.277.2.L423
13. Oue T, Yoneda A, Shima H, Taira Y, Puri P. Increased vascular endothelial growth factor peptide and gene expression in hypoplastic lung in nitrofen induced congenital diaphragmatic hernia in rats. *Pediatr Surg Int.* (2002) 18:221–6. doi: 10.1007/s003830100625
14. Galié N, Manes A, Branzi A. The endothelin system in pulmonary arterial hypertension. *Cardiovasc Res.* (2004) 61:227–37. doi: 10.1016/j.cardiores.2003.11.026
15. Hofmann AD, Friedmacher F, Takahashi H, Hunziker M, Gosemann JH, Puri P. Decreased apelin and apelin-receptor expression in the pulmonary vasculature of nitrofen-induced congenital diaphragmatic hernia. *Pediatr Surg Int.* (2014) 30:197–203. doi: 10.1007/s00383-013-3450-1
16. Montalva L, Antounians L, Zani A. Pulmonary hypertension secondary to congenital diaphragmatic hernia: factors and pathways involved in pulmonary vascular remodeling. *Pediatr Res.* (2019) 85:754–68. doi: 10.1038/s41390-019-0345-4
17. Putnam LR, Tsao K, Morini F, Lally PA, Miller CC, Lally KP, et al. Evaluation of variability in inhaled nitric oxide use and pulmonary hypertension in patients with congenital diaphragmatic hernia. *JAMA Pediatr.* (2016) 170:1188–94. doi: 10.1001/jamapediatrics.2016.2023
18. Ferguson DM, Gupta VS, Lally PA, Luco M, Tsao K, Lally KP, et al. Early, postnatal pulmonary hypertension severity predicts inpatient outcomes in congenital diaphragmatic hernia. *Neonatology.* (2021) 118:147–54. doi: 10.1159/000512966
19. Brooks H, Kirk ES, Vokonas PS, Urschel CW, Sonnenblick EH. Performance of the right ventricle under stress: relation to right coronary flow. *J Clin Invest.* (1971) 50:2176–83. doi: 10.1172/JCI106712
20. Jain A, Giesinger RE, Dakshinamurti S, ElSayed Y, Jankov RP, Weisz DE, et al. Care of the critically ill neonate with hypoxemic respiratory failure and acute pulmonary hypertension: framework for practice based on consensus opinion of neonatal hemodynamics working group. *J Perinatol.* (2022) 42:3–13. doi: 10.1038/s41372-021-01296-z
21. Byrne FA, Keller RL, Meadows J, Miniati D, Brook MM, Silverman NH, et al. Severe left diaphragmatic hernia limits size of fetal left heart more than does right diaphragmatic hernia. *Ultrasound Obstet Gynecol.* (2015) 46:688–94. doi: 10.1002/uog.14790
22. Mandell EW, Kinsella JP. Left ventricular dysfunction and persistent perfusion abnormalities in infants with congenital diaphragmatic hernia. *J Pediatr.* (2020) 219:7–8. doi: 10.1016/j.jpeds.2019.11.013
23. Shiyanagi S, Okazaki T, Shoji H, Shimizu T, Tanaka T, Takeda S, et al. Management of pulmonary hypertension in congenital diaphragmatic hernia: nitric oxide with prostaglandin-E1 versus nitric oxide alone. *Pediatr Surg Int.* (2008) 24:1101–4. doi: 10.1007/s00383-008-2225-6
24. Schwartz SM, Vermilion RP, Hirschl RB. Evaluation of left ventricular mass in children with left-sided congenital diaphragmatic hernia. *J Pediatr.* (1994) 125:447–51. doi: 10.1016/S0022-3476(05)83293-7
25. Siebert JR, Haas JE, Beckwith JB. Left ventricular hypoplasia in congenital diaphragmatic hernia. *J Pediatr Surg.* (1984) 19:567–71. doi: 10.1016/S0022-3468(84)80105-0
26. Le Duc K, Mur S, Sharma D, Aubry E, Recher M, Rakza T, et al. Prostaglandin E1 in infants with congenital diaphragmatic hernia (CDH) and life-threatening pulmonary hypertension. *J Pediatr Surg.* (2020) 55:1872–8. doi: 10.1016/j.jpedsurg.2020.01.008
27. Patel N, Massolo AC, Paria A, Stenhouse EJ, Hunter L, Finlay E, et al. Early postnatal ventricular dysfunction is associated with disease severity in patients with congenital diaphragmatic hernia. *J Pediatr.* (2018) 203:400–7. doi: 10.1016/j.jpeds.2018.07.062
28. Van Der Horst IW, Reiss I, Tibboel D. Therapeutic targets in neonatal pulmonary hypertension: linking pathophysiology to clinical medicine. *Expert Rev Respir Med.* (2008) 2:85–96. doi: 10.1586/17476348.2.1.85
29. Nair J, Lakshminrusimha S. Update on PPHN: mechanisms and treatment. *Semin Perinatol.* (2014) 38:78–91. doi: 10.1053/j.semperi.2013.11.004
30. Bialkowski A, Moenkemeyer F, Patel N. Intravenous sildenafil in the management of pulmonary hypertension associated with congenital diaphragmatic hernia. *Eur J Pediatr Surg.* (2015) 3:171–6. doi: 10.1055/s-0033-1357757
31. Lakshminrusimha S, Keszler M, Kirpalani H, Van Meurs K, Chess P, Ambalavanan N, et al. Milrinone in congenital diaphragmatic hernia–a randomized pilot trial: study protocol, review of literature and survey of current practices. *Matern Health Neonatol Perinatol.* (2017) 3:1–5. doi: 10.1186/s40748-017-0066-9
32. Skarda DE, Yoder BA, Anstadt EE, Lally PA, Greene T, McFadden M, et al. Epoprostenol does not affect mortality in neonates with congenital diaphragmatic hernia. *Eur J Pediatr Surg.* (2014) 25:454–9. doi: 10.1055/s-0034-1389096
33. Max M, Rossaint R. Inhaled prostacyclin in the treatment of pulmonary hypertension. *Eur J Pediatr.* (1999) 158:S23–6. doi: 10.1007/PL00014316
34. Mohamed WA, Ismail M, A. randomized, double-blind, placebo-controlled, prospective study of bosentan for the treatment of persistent pulmonary hypertension of the newborn. *J Perinatol.* (2012) 32:608–13. doi: 10.1038/jp.2011.157
35. Tonelli AR, Arelli V, Minai OA, Newman J, Bair N, Heresi GA, et al. Causes and circumstances of death in pulmonary arterial hypertension. *Am J Respir Crit Care Med.* (2013) 188:365–9. doi: 10.1164/rccm.201209-1640OC
36. Guihaire J, Bogaard H, Flécher E, Noly PE, Mercier O, Haddad F, et al. Experimental models of right heart failure: a window for translational research in pulmonary hypertension. *Semin Respir Crit Care Med.* (2013) 34:689–99. doi: 10.1055/s-0033-1355444
37. Julian FJ, Morgan DL, Moss RL, Gonzalez M, Dwivedi P. Myocyte growth without physiological impairment in gradually induced rat cardiac hypertrophy. *Circ Res.* (1981) 49:1300–10. doi: 10.1161/01.RES.49.6.1300

38. Gomez-Arroyo J, Mizuno S, Szczepanek K, van Tassell B, Natarajan R, dos Remedios CG, et al. Metabolic gene remodeling and mitochondrial dysfunction in failing right ventricular hypertrophy secondary to pulmonary arterial hypertension. *Circ Heart Fail.* (2013) 6:136–44. doi: 10.1161/CIRCHEARTFAILURE.111.966127
39. Drake JI, Bogaard HJ, Mizuno S, Clifton B, Xie B, Gao Y, et al. Molecular signature of a right heart failure program in chronic severe pulmonary hypertension. *Am J Respir Cell Mol Biol.* (2011) 45:1239–47. doi: 10.1165/rcmb.2010-0412OC
40. Voelkel NF, Bogaard HJ, Gomez-Arroyo J. The need to recognize the pulmonary circulation and the right ventricle as an integrated functional unit: facts and hypotheses (2013 Grover Conference series). *Pulm Circ.* (2015) 5:81–9. doi: 10.1086/679702
41. Urashima T, Zhao M, Wagner R, Fajardo G, Farahani S, Quertermous T, et al. Molecular and physiological characterization of RV remodeling in a murine model of pulmonary stenosis. *Am J Physiol Heart Circ Physiol.* (2008) 295:H1351–68. doi: 10.1152/ajpheart.91526.2007
42. Chua J, Zhou W, Ho JK, Patel NA, Mackensen GB, Mahajan A. Acute right ventricular pressure overload compromises left ventricular function by altering septal strain and rotation. *J Appl Physiol.* (2013) 115:186–93. doi: 10.1152/japplphysiol.01208.2012
43. Mendes-Ferreira P, Santos-Ribeiro D, Adão R, Maia-Rocha C, Mendes-Ferreira M, Sousa-Mendes C, et al. Distinct right ventricle remodeling in response to pressure overload in the rat. *Am J Physiol Heart Circ Physiol.* (2016) 311:H85–95. doi: 10.1152/ajpheart.00089.2016
44. Borgdorff MA, Bartelds B, Dickinson MG, Steendijk P, de Vroomen M, Berger RM. Distinct loading conditions reveal various patterns of right ventricular adaptation. *Am J Physiol Heart Circ Physiol.* (2013) 305:H354–64. doi: 10.1152/ajpheart.00180.2013
45. Rain S, Handoko ML, Trip P, Gan CTJ, Westerhof N, Stienen GJ, et al. Right ventricular diastolic impairment in patients with pulmonary arterial hypertension. *Circulation.* (2013) 128:2016–25. doi: 10.1161/CIRCULATIONAHA.113.001873
46. Jang S, Vanderpool RR, Avazmohammadi R, Lapshin E, Bachman TN, Sacks M, et al. Biomechanical and hemodynamic measures of right ventricular diastolic function: Translating tissue biomechanics to clinical relevance. *J Am Heart Assoc.* (2017) 6:e006084. doi: 10.1161/JAHA.117.006084
47. Aggarwal S, Stockman PT, Klein MD, Natarajan G. The right ventricular systolic to diastolic duration ratio: a simple prognostic marker in congenital diaphragmatic hernia? *Acta Paediatr.* (2011) 100:1315–8. doi: 10.1111/j.1651-2227.2011.02302.x
48. Patel N, Mills JF, Cheung MMH. Assessment of right ventricular function using tissue Doppler imaging in infants with pulmonary hypertension. *Neonatology.* (2009) 96:193–9. doi: 10.1159/000215585
49. Patel N, Lally PA, Kipfmueller F, Massolo AC, Luco M, van Meurs KP, et al. Ventricular dysfunction is a critical determinant of mortality in congenital diaphragmatic hernia. *Am J Respir Crit Care Med.* (2019) 200:1522–30. doi: 10.1164/rccm.201904-0731OC
50. Moenkemeyer F, Patel N. Right ventricular diastolic function measured by tissue Doppler imaging predicts early outcome in congenital diaphragmatic hernia. *Pediatr Critical Care Med.* (2014) 15:49–55. doi: 10.1097/PCC.0b013e31829b1e7a
51. Avitabile CM, Wang Y, Zhang X, Griffis H, Saavedra S, Adams S, et al. Right ventricular strain, brain natriuretic peptide, and mortality in congenital diaphragmatic hernia. *Ann Am Thorac Soc.* (2020) 17:1431–9. doi: 10.1513/AnnalsATS.201910-767OC
52. Reese J, Veldman A, Shah L, Vucovich M, Cotton RB. Inadvertent relaxation of the ductus arteriosus by pharmacologic agents that are commonly used in the neonatal period. *Semin Perinatol.* (2010) 34:222–30. doi: 10.1053/j.semperi.2010.02.007
53. Coceani F, Olley PM. The response of the ductus arteriosus to prostaglandins. *Can J Physiol Pharmacol.* (1973) 51:220–5. doi: 10.1139/y73-031
54. Elliott RB, Starling MB, Neutze JM. Medical manipulation of the ductus arteriosus. *Lancet.* (1975) 305:140–2. doi: 10.1016/S0140-6736(75)91432-4
55. Olley PM, Coceani F, Bodach E. E-type prostaglandins: a new emergency therapy for certain cyanotic congenital heart malformations. *Circulation.* (1976) 53:728–31. doi: 10.1161/01.CIR.53.4.728
56. Roehl SL, Townsend RJ. Alprostadil (Prostin VR pediatric sterile solution, the Upjohn Company). *Drug Intell Clin Pharm.* (1982) 16:823–32. doi: 10.1177/106002808201601103
57. Blanc J, Vouhé P, Bonnet D. Potts shunt in patients with pulmonary hypertension. *N Engl J Med.* (2004) 350:623. doi: 10.1056/NEJM200402053500623
58. Aggarwal M, Grady RM, Choudhry S, Anwar S, Eghtesady P, Singh GK. Potts shunt improves right ventricular function and coupling with pulmonary circulation in children with suprasystemic pulmonary arterial hypertension. *Circ Cardiovasc Imaging.* (2018) 11:e007964. doi: 10.1161/CIRCIMAGING.118.007964
59. Graham EM, Bradley SM, Atz AM. Preoperative management of hypoplastic left heart syndrome. *Expert Opin Pharmacother.* (2005) 6:687–93. doi: 10.1517/14656566.6.5.687
60. Naka Y, Roy DK, Liao H, Chowdhury NC, Michler RE, Oz MC, et al. cAMP-mediated vascular protection in an orthotopic rat lung transplant model: Insights into the mechanism of action of prostaglandin E1 to improve lung preservation. *Circ Res.* (1996) 79:773–83. doi: 10.1161/01.RES.79.4.773
61. SWAN PK TIBBALLS J, DUNCAN AW. Prostaglandin E1 in primary pulmonary hypertension. *Crit Care Med.* (1986) 14:72–3. doi: 10.1097/00003246-198601000-00019
62. Fortas F, Di Nardo M, Yousef N, Humbert M, De Luca D. Life-threatening PPHN refractory to nitric oxide: proposal for a rational therapeutic algorithm. *Eur J Pediatr.* (2021) 180:2379–87. doi: 10.1007/s00431-021-04138-4
63. Gupta N, Kamlin CO, Cheung M, Stewart M, Patel N. Prostaglandin E1 use during neonatal transfer: potential beneficial role in persistent pulmonary hypertension of the newborn. *Arch Dis Child Fetal Neonatal Ed.* (2013) 98:F186–8. doi: 10.1136/archdischild-2012-303294
64. Sakuma F, Miyata M, Kasukawa R. Suppressive effect of prostaglandin E1 on pulmonary hypertension induced by monocrotaline in rats. *Lung.* (1999) 177:77–88. doi: 10.1007/PL00007632
65. Ono S, Tanita T, Hoshikawa Y, Song C, Maeda S, Tabata T, et al. Effects of prostaglandin E1 (PGE1) on pulmonary hypertension and lung vascular remodeling in a rat monocrotaline model of human pulmonary hypertension. *Jpn J Thorac Dis.* (1995) 33:862–7. doi: 10.1097/00024382-199512001-00182
66. Akkinapally S, Hundalani SG, Kulkarni M, Fernandes CJ, Cabrera AG, Shivanna B, et al. Prostaglandin E1 for maintaining ductal patency in neonates with ductal-dependent cardiac lesions. *Cochrane Database Syst Rev.* (2018) CD011417. doi: 10.1002/14651858.CD011417.pub2
67. Pierro M, Thebaud B. Understanding and treating pulmonary hypertension in congenital diaphragmatic hernia. *Semin Fetal Neonat Med.* (2014) 19:357–63. doi: 10.1016/j.siny.2014.09.008
68. Inamura N, Kubota A, Nakajima T, Kayatani F, Okuyama H, Oue T, et al. A proposal of new therapeutic strategy for antenatally diagnosed congenital diaphragmatic hernia. *J Pediatr Surg.* (2005) 40:1315–9. doi: 10.1016/j.jpedsurg.2005.05.018
69. Inamura N, Kubota A, Ishii R, Ishii Y, Kawazu Y, Hamamichi Y, et al. Efficacy of the circulatory management of an antenatally diagnosed congenital diaphragmatic hernia: outcomes of the proposed strategy. *Pediatr Surg Int.* (2014) 30:889–94. doi: 10.1007/s00383-014-3574-y
70. Lawrence KM, Berger K, Herkert L, Franciscovich C, O'Dea CLH, Waqar LN, et al. Use of prostaglandin E1 to treat pulmonary hypertension in congenital diaphragmatic hernia. *J Pediatr Surg.* (2019) 54:55–9. doi: 10.1016/j.jpedsurg.2018.10.039
71. Koch A, Singer H. Normal values of B type natriuretic peptide in infants, children, and adolescents. *Heart.* (2003) 89:875–8. doi: 10.1136/heart.89.8.875
72. Hofmann SR, Stadler K, Heilmann A, Häusler HJ, Fitze G, Kamin G, et al. Stabilisation of cardiopulmonary function in newborns with congenital diaphragmatic hernia using lung function parameters and hemodynamic management. *Klin Padiatr.* (2012) 224:e1-0. doi: 10.1055/s-0031-1299731
73. Buss M, Williams G, Dilley A, Jones O. Prevention of heart failure in the management of congenital diaphragmatic hernia by maintaining ductal patency. A case report. *J Pediatr Surg.* (2006) 41:e9-11. doi: 10.1016/j.jpedsurg.2006.01.003
74. Filan PM, McDougall PN, Shekerdemian LS. Combination pharmacotherapy for severe neonatal pulmonary hypertension. *J Paediatr Child Health.* (2006) 42:219–20. doi: 10.1111/j.1440-1754.2006.00835.x

75. Divekar A, Seshia MM, Kesselman M. Non-restrictive ductal patency in management of cardiac failure in congenital diaphragmatic hernia-non-invasive biventricular assist. *J Neonat Biol*. (2015) 4:171. doi: 10.4172/2167-0897.1000171
76. Aljohani OA, Rao R, Mackie D, Haldeman S, Karamlou T. Combination of PGE1 and pulmonary vasodilator therapy in managing a challenging case of severe PAH secondary to CDH. *World J Pediatr Congenit Heart Surg*. (2020) 11:525–7. doi: 10.1177/2150135120912678
77. Snoek KG, Reiss IKM, Greenough A, Capolupo I, Urlesberger B, Wessel L, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH EURO Consortium Consensus - 2015 Update. *Neonatology*. (2016) 110:66–74. doi: 10.1159/000444210
78. Puligandla PS, Skarsgard ED, Offringa M, Adatia I, Baird R, Bailey JM, et al. Diagnosis and management of congenital diaphragmatic hernia: a clinical practice guideline. *CMAJ*. (2018) 190:E103–12. doi: 10.1503/cmaj.170206
79. Ruano R, Lazar DA, Cass DL, Zamora IJ, Lee TC, Cassady CI, et al. Fetal lung volume and quantification of liver herniation by magnetic resonance imaging in isolated congenital diaphragmatic hernia. *Ultrasound Obstet Gynecol*. (2014) 43:662–9. doi: 10.1002/uog.13223
80. van den Hout L, Tibboel D, Vijfhuize S. te Beest H, Hop W, Reiss I. The VICI-trial: high frequency oscillation versus conventional mechanical ventilation in newborns with congenital diaphragmatic hernia: an international multicentre randomized controlled trial. *BMC Pediatr*. (2011) 11:1–7. doi: 10.1186/1471-2431-11-98
81. Deprest JA, Benachi A, Gratacos E, Nicolaides KH, Berg C, Persico N, et al. Randomized trial of fetal surgery for moderate left diaphragmatic hernia. *N Engl J Med*. (2021) 385:119–29. doi: 10.1056/NEJMoa2026983
82. Neonatal Inhaled Nitric Oxide Study Group. Inhaled nitric oxide and hypoxic respiratory failure in infants with congenital diaphragmatic hernia. *Pediatrics*. (1997) 99:838–45. doi: 10.1542/peds.99.6.838
83. Cochius-Den Otter S, Schaible T, Greenough A, van Heijst A, Patel N, Allegaert K, Van Rosmalen J, Tibboel D. The CoDiNOS trial protocol: an international randomised controlled trial of intravenous sildenafil versus inhaled nitric oxide for the treatment of pulmonary hypertension in neonates with congenital diaphragmatic hernia. *BMJ Open*. (2019) 9:e032122. doi: 10.1136/bmjopen-2019-032122
84. Wright JCE, Budd JLS, Field DJ, Draper ES. Epidemiology and outcome of congenital diaphragmatic hernia: a 9-year experience. *Paediatr Perinat Epidemiol*. (2011) 25:144–9. doi: 10.1111/j.1365-3016.2010.01172.x

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EDITED BY
Hannes Sallmon,
Deutsches Herzzentrum Berlin, Germany

REVIEWED BY
Jason Gien,
University of Colorado Anschutz Medical Campus, United States
Neysan Rafat,
University of Heidelberg, Germany
Paul D. Losty,
University of Liverpool, United Kingdom
Christe Weiss,
University of Heidelberg, Germany

*CORRESPONDENCE
Neil Patel
neil.patel@ggc.scot.nhs.uk

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The heart in congenital diaphragmatic hernia: Knowns, unknowns, and future priorities

Neil Patel[1]*, Anna C. Massolo[2], Ulrike S. Kraemer[3] and Florian Kipfmueller[4]

[1]Department of Neonatology, Royal Hospital for Children, Glasgow, United Kingdom, [2]Ospedale San Filippo Neri, Rome, Italy, [3]Intensive Care Unit, Department of Pediatric Surgery, Erasmus MC-Sophia Children's Hospital, Rotterdam, Netherlands, [4]Department of Neonatology and Pediatric Intensive Care, Children's Hospital, University of Bonn, Bonn, Germany

There is growing recognition that the heart is a key contributor to the pathophysiology of congenital diaphragmatic hernia (CDH), in conjunction with developmental abnormalities of the lung and pulmonary vasculature. Investigations to date have demonstrated altered fetal cardiac morphology, notably relative hypoplasia of the fetal left heart, as well as early postnatal right and left ventricular dysfunction which appears to be independently associated with adverse outcomes. However, many more unknowns remain, not least an understanding of the genetic and cellular basis for cardiac dysplasia and dysfunction in CDH, the relationship between fetal, postnatal and long-term cardiac function, and the impact on other parts of the body especially the developing brain. Consensus on how to measure and classify cardiac function and pulmonary hypertension in CDH is also required, potentially using both non-invasive imaging and biomarkers. This may allow routine assessment of the relative contribution of cardiac dysfunction to individual patient pathophysiological phenotype and enable better, individualized therapeutic strategies incorporating targeted use of fetal therapies, cardiac pharmacotherapies, and extra-corporeal membrane oxygenation (ECMO). Collaborative, multi-model approaches are now required to explore these unknowns and fully appreciate the role of the heart in CDH.

KEYWORDS

congenital diaphragmatic hernia, ventricular function, ventricular hypoplasia, pulmonary hypertension, echocardiography, biomarkers, cardiac

Introduction

The past decade has seen growing appreciation of the heart as a key component of disease pathophysiology in congenital diaphragmatic hernia (CDH) (1). The application of advanced imaging modalities combined with multi-center registry analysis has shone new light on fetal cardiac development and the role of postnatal cardiac function, alongside pulmonary hypertension and pulmonary hypoplasia, in determining clinical phenotypes and outcomes in CDH. However, there is much more to understand

in relation to cardiac development, mechanisms of dysfunction, and their clinical significance throughout life. Addressing these uncertainties may lead to new therapeutic strategies and improved outcomes in CDH.

This review aims to provide a current "state of the art," comprehensively reviewing what is known of the heart from fetal life to adulthood in CDH, highlighting the major areas of ongoing uncertainty, and identifying key priorities for further investigation.

The fetal heart in congenital diaphragmatic hernia

Cardiac abnormalities in CDH, as with those in the lungs and pulmonary circulation, undoubtably have their origins in the fetus.

Congenital heart disease in fetal congenital diaphragmatic hernia

Congenital diaphragmatic hernia and congenital heart disease (CHD) are frequently associated. In recent systematic review up to 15% of live born CDH patients also have CHD, though rates may be as high as 28% when stillborn and terminated CDH cases are included (2). Conversely, 0.3% of infants requiring surgery for CHD have associated CDH (3).

Forty-two percent of infants with CDH and CHD are considered to have critical lesions, rather than simple shunts (ventricular and atrial septal defects, patent ductus arteriosus). The commonest associated cardiac lesions are, in order of frequency, ventricular and atrial septal defects, hypoplastic left heart syndrome (HLHS), coarctation of the aorta or aortic hypoplasia, tetralogy of Fallot and double outlet right ventricle (2, 4).

The relationship between measures of CDH severity (e.g., defect size, abdominal organ position, fetal lung size) and CHD incidence is as not yet understood. Similarly, the potential shared mechanisms, including genetic and environmental factors, contributing to CDH and associated CHD remain unclear, but may involve disruption of common pathways in cardiac and diaphragmatic development (5, 6). An increasing number of genetic mutations have been identified in CDH, however, the frequency of these is not affected by the presence or absence of associated anomalies including CHD (7, 8). Of note, HLHS and aortic anomalies may represent one end of a spectrum of left heart hypoplasia in CDH that is distinct from other mechanisms of CHD, as discussed in more detail below.

The presence of any CHD significantly affects surgical management of the diaphragmatic hernia. Systematic review indicates that CDH repair rates are lower (72% vs. 85%), patch repair more frequent (45% vs. 30%), and minimally invasive approaches are employed less often (5% vs. 17%). CDH repair typically precedes any cardiac intervention, and notably only 10% of affected cases received a cardiac intervention during the neonatal period (2). ECMO use is similar between CHD and non-CHD groups (9). The commonest cardiac surgeries performed in CDH cases are hybrid procedures, coarctation and aortic arch repair, VSD repair and pulmonary artery banding (3).

Importantly, any CHD confers lower survival rates in CDH, which approach 50% overall and as low as 30% for infants with critical cardiac lesions, and 1–5% for infants with CDH and HLHS (2, 3, 9, 10). Conversely, the presence of CDH also confers higher overall and peri-operative mortality when compared to all CHD, as well as increased rates of post-op complications and longer length of stay after cardiac surgery in both high and low risk cardiac lesions (3, 4).

Though no formal guidelines exist for management of CHD in CDH, an algorithmic, team approach has been advocated to assist in complex decision-making for critical lesions (11).

The remainder of this review will now focus on abnormalities of cardiac structure and function distinct from classical CHD, and which appear to be specific to CDH pathophysiology.

Cardiac hypoplasia in congenital diaphragmatic hernia

Hypoplasia of the developing heart in CDH is an established finding, observed first in post-mortem studies and confirmed by echocardiography analyses, **Table 1** (12–14).

In fetuses with left-sided CDH ventricular hypoplasia appears to predominantly affect the left ventricle (LV), characterized by reduced ventricular width and associated reductions in LV area and mass, together with reduced aortic valve diameter (14, 15). Right ventricular (RV) dimensions may also be reduced at earlier gestation but appear to increase, along with pulmonary artery diameter, at later gestation (16, 17). Accordingly, ratios of left to right cardiac dimensions in left-sided CDH are lowest at later gestations (18–20). Conversely, in right-sided CDH the limited available data indicate reduced fetal right ventricular and pulmonary arterial dimensions combined and less severe LV hypoplasia than in left-sided CDH (14, 21).

Multiple mechanisms of fetal cardiac hypoplasia have been proposed, though the relative contribution and timing of these remains uncertain (**Figure 1**):

1. Mechanical compression of the developing heart by herniating abdominal contents. Consistent with this hypothesis ventricular hypoplasia is greatest on the ipsilateral side and appears to disproportionately affects ventricular width rather than length (14, 18). Analogous reductions in fetal mitral valve and AV diameter are observed in fetuses with large, compressive left-sided congenital lung masses (22).

TABLE 1 Echocardiographic studies of fetal and early postnatal cardiac dimensions and fetal cardiac function in CDH.

References	*N*	Gestation/postnatal age	Fetal heart dimensions	Neonatal dimensions	Outcome
Schwartz et al. (33)	20 L CDH	"On [neonatal] admission"	–	Lower LV mass in CDH vs. controls	LV mass lower in cases who required ECMO
Thebaud et al. (19)	40 fetal 32 newborn CDH	21–30 and 31–40 weeks	Reduced LV:RV (MV:TV and Ao:PA) ratios at 31–40 weeks	–	LV:RV at 31–40 weeks correlated with non-survival and PH.
Baumgart et al. (16)	23 newborn CDH	38–40 weeks	–	Reduced Ao, LV mass, MV diameter and increased PV diameter in CDH.	LV mass lower in non-survivors
VanderWall et al. (17)	12 CDH fetus	17–25 weeks	Reduced RV (TV) and LV (MV) width, LV volume and mass in CDH.	–	No difference in fetal dimensions in survivors vs. non-survivors
Van Mieghem et al. (27)	27 fetal L CDH, 117 controls	–	LV ED dia smaller in CDH. No difference in LV function (EF, FS, MPI) in CDH vs. controls.	–	FETO did not affect cardiac size but reduced MPI. Reversal of FETO did not affect cardiac size or function.
Stressig et al. (24)	32 CDH fetuses	19–39 weeks	Reduced *z*-score of MV, Ao valve, MV:TV, Ao/PA in cases with ductus venosus and IVC streaming to right heart	–	
Vogel et al. (15)	125 111 L CDH 14 R CDH	24 (17–39) weeks	Age-adjusted AV, MV, LV length, LV volume, were all smaller in CDH	*Z*-scores of left heart structures increased from prenatal to postnatal echo	No association between prenatal left heart *Z*-scores and postnatal survival
DeKoninck et al. (21)	17 R CDH fetus, 17 controls	27 (24–29) weeks	Reduced PV, RV ED and RV ES diameters, RVO and RV SV in CDH. No difference in AoV and LV dimensions or MPI.	–	–
DeKoninck et al. (151)	38 fetuses, 29 L CDH 9 R CDH	27 (21–32) weeks	Increased LV strain in CDH, no correlation with O:E LHR	–	–
Yamoto et al. (32)	99 controls, 33 CDH fetus	Control 32 (17–39) CDH 32 (21–40) weeks	Cardiothoracic area (CTAR) ratio, MPA:Ao, TV:MV all s altered in CDH, before and after 32 weeks gestation	–	CTAR, MPA:Ao and TV:MV all differentiated survivors vs. non-survivors. TV:MV had greatest sensitivity
Byrne et al. (14)	188 fetuses, 171 L CDH, 17 R CDH	16–37 weeks	MV, AV, LV volume and LVO, reduced in "severe CDH" (LHR < 1 and liver in chest in L CDH).	–	
Degenhardt et al. (25)	8 CDH fetus pre and post FETO		No significant change in function (TAPSE, MAPSE, MPI) pre and post FETO	–	–
Kailin et al. (31)	52 L CDH fetus	27 ± 5 weeks and earliest postnatal echo	–	AV and LV SAX dimension *z*-scores significantly lower prenatally vs. postnatally	Fetal AV *z*-score independently associated with iNO use
Lemini et al. (28)	31 L CDH fetus, 75 controls	34 ± 6 weeks	Impaired diastolic function in fetal CDH assessed by tissue Doppler imaging.	–	–
Kaya et al. (29)	28 CDH 20 L CDH 8 R CDH. 56 controls		RV parameters only. No difference in RV TDI velocities. Increased ICT, IRT and RV MPI in CDH	–	–
Coffman et al. (30)	52 infants, 40 L CDH, 12 R CDH	Birth – 1 month of age	–	Reduced *z*-scores for LVIDd, LVIDs, aortic annulus, arch, sino-tubular junction	Length of stay inversely correlated with left heart structures
Massolo et al. (18)	12 L CDH fetus, 41 controls	24–26 weeks, 30–32 weeks, 34–36 weeks	Reduced MV, LV area, TV and RV area, MV:TV at 24–26 weeks. At 34–36 weeks reduced MV, LV area, and MV:TV.	–	MV and MV *z*-score at 24-26 weeks associated with death/ECMO

LV, left ventricle; RV, right ventricle; MV, mitral valve; TV, tricuspid valve; Ao, aortic diameter; PA, pulmonary artery diameter; LVO, left ventricular output; EF, ejection fraction; FS, fractional shortening; MPI, myocardial performance index; FETO, fetal endoscopic tracheal occlusion; ED, end diastolic; ES, end systolic; SV, stroke volume; AV, aortic valve; SAX, short axis; TDI, tissue Doppler imaging; MPA, main pulmonary artery; ICT, isovolumic contraction time; IRT, isovolumic relaxation time; LVID, LV internal diameter.

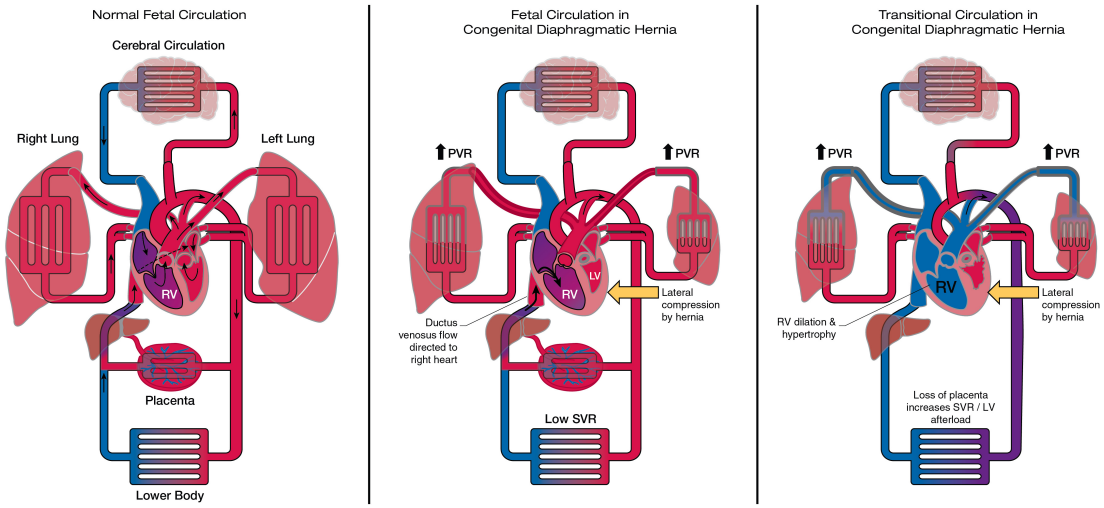

FIGURE 1
The heart and circulation in CDH during the fetal and transitional period. In the fetal CDH circulation LV hypoplasia may be related to redirection of ductus venosus flow to the right heart and reduced pulmonary blood flow, together with lateral compression by the herniating abdominal contents. In the transitional period removal of the placenta increases afterload on the ventricles. The right ventricle dilates and becomes dysfunctional in the face of sustained postnatal increase in PVR. LV function is at risk due to pre-existent hypoplasia, septal displacement and the acute increase in afterload. RV, right ventricle; LV, left ventricle; PVR, pulmonary vascular resistance; SVR, systemic vascular resistance.

2. Reduced pulmonary blood flow in CDH compared to normal fetuses, due to the primary structural changes in the pulmonary vasculature characteristic of CDH. This in turn leads to reduced pulmonary venous return, LV filling and growth. In support of this flow-based mechanism reduced LV dimensions are similarly observed in fetuses with anomalous pulmonary venous drainage (23). This mechanism would in theory have greatest impact later in gestation when, in the normal fetus, increases in fetal pulmonary blood flow and LV size are usually observed.
3. An additional flow-based mechanism of LV hypoplasia in CDH may be due to mediastinal shift and liver herniation resulting in re-direction of ductus venosus (DV) and inferior vena cava (IVC) streaming away from the foramen ovale and resulting in reduced LV filling and growth (24). Oxygenated venous return is instead re-directed to the right heart and thereafter the majority will pass across the patent ductus and a minority to the pulmonary arteries. Increased oxygenation of the fetal pulmonary blood flow, as a secondary consequence of this mechanism, has also been hypothesized as a possible cause of the excessive muscularization of the pulmonary arterioles in CDH.

Fetal cardiac function in congenital diaphragmatic hernia

Prenatal cohort studies of myocardial function have not to date demonstrated significant fetal cardiac dysfunction (25–29). Theoretically, the fetal circulation may mitigate against cardiac dysfunction *in utero*; the presence of a patent ductus and low resistance placenta protecting the RV from excessive afterload (despite increased resistance in the pulmonary vasculature) and ensure that the non-dominant, hypoplastic LV remains untaxed by excessive preload or afterload, **Figure 1**. Prospective studies throughout gestation are needed to understand the natural history and clinical significance of fetal cardiac function.

Clinical significance of fetal cardiac hypoplasia in congenital diaphragmatic hernia

The relationship between fetal cardiac hypoplasia and clinical outcome remains unresolved. In single center cohorts of left-sided CDH left heart dimensions, notably LV width, mitral and aortic valve diameters, and ratios of left to right-sided dimensions have been associated with adverse outcome including higher neonatal mortality, higher ECMO use, increased inhaled nitric oxide use, and prolonged length of stay (18, 19, 30–33).

However, these findings have not been replicated in other series. VanderWall did not observe any association between fetal ventricular dimensions and outcome, though small cohort size may have been a factor (17). Vogel et al. observed mild to moderate fetal LV hypoplasia in a cohort of 125 CDH cases. LV fetal dimensions, expressed as *z*-scores, were not associated with postnatal survival when analyzed as continuous

data, but were on categorical analysis (15). In the same cohort, there was a trend toward normalization of LV dimensions on paired postnatal, post-CDH repair, echocardiograms. This may suggest that reduced LV volumes are recruitable in response to postnatal hemodynamics.

Further investigation is a priority to determine which, if any, fetal cardiac dimensions have the greatest prognostic utility, when and how these should be measured, and the mechanisms by which these might directly influence postnatal LV dimensions, function, and outcome.

Cellular and metabolic function in the fetal heart in congenital diaphragmatic hernia

Whether structural changes in the fetal heart in CDH are associated with primary or secondary abnormalities at a genetic, epigenetic, cellular or metabolic level remains largely unknown. Cardiac hypoplasia in nitrofen rat models of CDH is associated with reduced expression of insulin-like growth factor-1, epidermal growth factor, basic fibroblast growth factor and platelet derived growth factor, **Table 2** (34, 35). Zhaourigetu et al. also recently observed abnormal cardiomyocyte structure associated with reduced expression of mitochondrial and fatty acid biogenesis genes in this CDH model (36).

A single post-mortem study of hearts from human CDH fetuses also demonstrated dis-homogenous growth factor expression (37). In the same study fetal CDH hearts had abnormal thickening and proliferation of intra-myocardial vessels, particularly in the inter-ventricular septum. This raises the hypothesis that intra-cardiac vasculature in CDH might demonstrate developmental abnormalities analogous to those in the pulmonary vasculature (18).

In fetal rabbit models of CDH, though not lamb models, cardiac hypoplasia is associated with decreased ventricular wall thickness and increased septal thickness (38, 39). Ventricular wall thickening, due to cardiomyocyte hyperplasia, is also observed in hypoplastic left heart syndrome (HLHS), potentially pointing to common mechanisms of cardiac dysgenesis in these two conditions (40).

Fetal therapies and the developing heart in congenital diaphragmatic hernia

In limited cohort studies fetal tracheal occlusion (FETO), the principal prenatal therapy attempted for CDH, does not appear to significantly affect fetal cardiac dimensions or function in CDH (16, 18). Of note, the recent larger multicenter TOTAL trials of FETO did not include assessment of cardiac dimensions or function FETO (41).

A variety of pre-natal pharmacotherapies aimed at modulating pulmonary vascular development have been investigated in pre-clinical CDH models (42). Sildenafil, a phosphodiesterase 5 inhibitor partially reverses pulmonary vascular abnormalities and lowering pulmonary vascular resistance and reducing RV hypertrophy in animal models (43–45). However, adverse events associated with of prenatal administration in non-CDH patients have prevented clinical trials in diaphragmatic hernia (46). Maternal hyperoxygenation may be an alternative therapeutic approach which in congenital heart disease has been observed to increase pulmonary blood flow, and could potentially increase left ventricular flow and size in turn (47, 48). Further investigation is required of the impact of prenatal therapies on cardiac development and postnatal function in CDH.

TABLE 2 Studies of fetal cardiac cellular structure and metabolism in CDH.

References	Experimental CDH model	Findings
Karamanoukian et al. (38)	Fetal lamb	No difference in ventricular wall thickness, total protein, DNA collagen, and elastin between CDH and controls
Tannuri (39)	Fetal rabbit	Decreased ventricular wall thickness, increased septal thickness.
Teramoto and Puri (34)	Nitrofen rat	Decreased insulin like growth factor-1 (IGF-1) and epidermal growth factor (EGF) expression in CDH hearts associated with cardiac hypoplasia.
Guarino et al. (35)	Nitrofen rat	Expression of basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF) was significantly reduced in CDH heart, with associated reduced heart growth.
Baptista et al. (152)	Nitrofen rat	Significant oscillation in BNP and angiotensin mRNA in nitrofen exposed pups compared to controls, but not in CDH specifically.
Pelizzo et al. (37)	Post mortem 7 human CDH fetuses	Dis-homogenous growth factor distribution in ventricles in fetal CDH. Increased small intramyocardial artery density and increased vascular thickness in ventricular walls.
Zambaiti et al. (153)	Fetal lamb	Early tracheal occlusion was associated with LV myocardial enlargement, increased endothelin-1 (ET-1) and transforming growth factor beta (TGF beta) expression.
Zhaorigetu et al. (36)	Nitrofen rat	Increased ventricular myocyte hypoxia, downregulation of mitochondrial and fatty acid biogenesis genes. Altered mitochondrial structure.

The heart in early postnatal life in congenital diaphragmatic hernia

Changes in the fetal heart in CDH may be important precedents of postnatal cardiac function. Recent investigation using functional echocardiography and multi-center registry analysis have led to new, but still incomplete, understanding of the heart's unsteady transition from pre-natal to post-natal environment in CDH, **Table 3**. **Figure 1** provides a visual overview of these changes in loading conditions, cardiac morphology and function from fetal to postnatal life.

Right ventricle dysfunction in congenital diaphragmatic hernia

Dysfunction in the RV, in conjunction with ventricular dilatation and hypertrophy, has been demonstrated from the first days of life in CDH (49). These are considered to result from pathological increases in RV afterload as a result of the structural and functional pulmonary vascular abnormalities characteristic of CDH (50). Increased RV pressure, and a concomitant reduced systemic pressure, may also reduce coronary artery flow gradient, leading to RV ischemia and dysfunction. Whilst recognized in other pulmonary hypertensive disease this mechanism has not been studied directly in CDH (51).

Early RV dysfunction in CDH is characterized by both impaired systolic function and, notably early diastolic dysfunction, demonstrated by reduced diastolic myocardial velocities and shortened diastolic duration in the RV (52–55).

Right ventricle dysfunction, when present, likely contributes to early clinical instability *via* a number of mechanisms. First, the failing RV may become "uncoupled" from the pulmonary circulation, unable to maintain adequate pulmonary blood flow, contributing to impaired oxygenation, and reduced LV filling and output (56). Second, RV dysfunction negatively impacts LV performance *via* mechanisms of ventricular interdependence including shared myocardial fibers, disruption of normal systolic and diastolic time intervals, and septal displacement reducing LV volume (40). Through these mechanisms RV dysfunction may be a key mediator of adverse effects of pulmonary hypertension in CDH (57, 58).

In cohort studies early RV dysfunction is associated with prolonged duration of respiratory support, increased mortality and ECMO use (49, 59–61). In a recent large multi-center registry analysis of cardiac function in the first 48 h of life RV dysfunction was present in 34% of CDH cases either in isolation in combination with LV dysfunction, and was associated with increased mortality and ECMO use (62).

TABLE 3 Early postnatal cardiac function in CDH.

References	Population	Parameter	Ventricular function and relationship to outcome
Patel et al. (54)	9 CDH, 28 controls	RV MPI	Reduced RV MPI in CDH
Patel et al. (53)	11 CDH infants median 18 days. 28 controls.	TDI myocardial velocities and TV Doppler velocities	Reduced RV early diastolic velocities in CDH.
Aggarwal et al. (154)	29 CDH, 27 controls. <3 days	Systolic:Diastolic time durations	Reduced RV diastolic time intervals in CDH, and in CDH non-survivors.
Aggarwal et al. (52)	34 CDH, 35 controls	RV and LV MPI and cardiac index (CI)	Reduced RV and LV MPI, and CI in CDH compared to controls, and CDH cases who died/required ECMO. LV MPI and CI associated with mortality.
Moenkemeyer and Patel (49)	16 CDH infants (13 L CDH, 3 R CDH) day 1–2	TDI myocardial velocities	Reduced RV early diastolic myocardial velocities in non-survivors. RV diastolic dysfunction correlated with increased length of stay and duration of respiratory support
Altit et al. (60)	34 CDH, first 48 h.	STE-derived strain. RV FAC and TAPSE. EF.	Reduced RV and LV longitudinal strain and strain rate, RV TAPSE and FAC, and LV EF in CDH cases who required ECMO.
Patel et al. (63)	25 CDH (21 L CDH) and 20 controls in first 48 h of life	TDI and STE-derived strain	Global reduction in RV and LV systolic strain in CDH. LV longitudinal strain correlated with fetal lung volume, duration of intubation and length of stay, and was lower in non-survivors/ECMO.
Altit et al. (55)	44 CDH, 18 controls. First 48 h	Ventricular strain. RV FAC, TAPSE. LV EF, stroke distance	Reduced RV and LV longitudinal strain, reduced RV FAC and TAPSE, and LV stroke distance in CDH.
Naguib et al. (56)	20 CDH infants	RV outflow VTI	Lower RV output in CDH non-survivors.
Gaffar et al. (66)	27 CDH cases (21 L CDH)	RV and LV CI and VTI, LV EF	Lower LV CI in CDH cases who received ECMO.
Patel et al. (62)	1173 CDH infants, (971 L, 202 R). First 48 h of life	CDH Registry analysis. Cardiac function reported by 59 centers	Cardiac function normal in 61%, RV dysfunction in 15%, LV dysfunction in 5%, biventricular dysfunction in 19%. LV and biventricular dysfunction associated with increased mortality. RV and LV dysfunction associated with ECMO
Avitabile et al. (61)	220 CDH (184 L CDH).	RV strain, FAC, FWS pre-op, post op (<1 week) and recovery phase (>1 week)	Abnormal RV strain associated with ECMO use. Abnormal RV strain in recovery phase associated with increased mortality. Improvement in net RV strain after repair.

RV, right ventricle; MPI, myocardial performance index; TDI, tissue Doppler imaging; TV, tricuspid valve; CI, cardiac index; STE, speckle tracking echocardiography; FAC, fractional area change; TAPSE, tricuspid annular plane systolic excursion; EF, ejection fraction; VTI, velocity-time integer; EF, ejection fraction; FWS, fractional wall shortening.

Left ventricular dysfunction in congenital diaphragmatic hernia

The potential for postnatal left ventricular dysfunction in newborns with CDH has been long-recognized by experienced clinicians, and more recently quantified using functional echocardiographic techniques (52, 55, 63). Its importance as a component of CDH pathophysiology was highlighted by recent multi-center analysis demonstrating that LV dysfunction, in isolation or combined with RV dysfunction, independently predicted death and ECMO use (62).

Early postnatal LV dysfunction is frequent. In registry analysis of over 1100 CDH cases from 59 centers LV dysfunction was reported in 24% of CDH cases (62). However, this may be an underestimate; In smaller cohort studies utilizing sensitive, quantitative strain analysis of myocardial function LV dysfunction was observed in 56% of cases (63).

Postnatal LV dysfunction is characterized by both global systolic and diastolic dysfunction affecting longitudinal, circumferential and radial function, and dyssynchrony of myocardial segments (60, 63, 64). Postnatal LV volumes may also be reduced possibly due to compressive actions of a dilated RV and the herniated organs, combined with the legacy of fetal LV hypoplasia.

Left ventricle dysfunction is frequently observed in combination with RV dysfunction and may therefore be a secondary consequence *via* mechanisms of ventricular interdependence, as discussed above (64). However primary LV dysfunction may occur in the absence of, or disproportionate to, RV dysfunction (62). Multiple factors are hypothesized to contribute to primary postnatal LV dysfunction in the transitional period (1, 65):

I. Fetal LV dysfunction
II. Fetal LV hypoplasia
III. Changes in LV loading conditions: reduced preload, due to failure of normal increases in pulmonary blood flow at birth, and increased afterload due to removal of the low-resistance placenta from the systemic circulation
IV. Hypoxia and acidosis contributing to worsening ventricular function

Left ventricle dysfunction likely contributes to adverse clinical outcome *via* reduced LV output and systemic blood flow, resulting in impaired tissue oxygenation and a viscous cycle of worsening hypoxia and acidosis (66). Accordingly, the systemic hypotension frequently observed in early CDH is likely to be a consequence of impaired cardiac function and cardiac output, rather than hypovolemia or low systemic vascular resistance.

Early LV dysfunction is associated with other markers of CDH severity, including smaller fetal lung volumes, larger diaphragmatic defect size, and liver herniation (62, 63). However, Dao et al. have observed that LV dysfunction may also occur in "lower risk" CDH cases with smaller defects (67).

Of note, LV dysfunction appears to be a transitional phenomenon, apparently present from soon after birth but with the potential to improve rapidly over the first week of life (68, 69). This may have important consequences for individualized management strategies, including ECMO, as discussed below.

Left ventricle dysfunction as a mechanism of pulmonary hypertension

Left ventricle dysfunction leading to increased end diastolic, left atrial and pulmonary venous pressures is well-recognized as a mechanism of increased pulmonary vascular resistance in adult heart disease (70). There is increasing recognition that similar mechanisms may occur in CDH in the setting of early, transition LV dysfunction, contributing to a post-capillary increase in pulmonary venous resistance, distinct from pre-capillary changes in pulmonary arterial resistance (1). This may have important implications for targeted management strategies in CDH, including the suitability of pulmonary vasodilators, as discussed below.

Hemodynamic phenotypes in congenital diaphragmatic hernia

The complex interplay of ventriculo-arterial, inter-ventricular, and cardio-respiratory interactions in CDH results in dynamic hemodynamic phenotypes with variable RV and LV function and dysfunction. As discussed later, this concept may be important in targeted, individualized therapeutic strategies.

Atrial shunting patterns may help define these phenotypes in the clinical setting. As recently highlighted by Wehrmann et al., the frequent presence of left-to-right atrial shunting in CDH, despite elevated pulmonary artery pressures, should prompt a closer examination of the left ventricular size and function (71).

Variability in cardiac dysfunction and clinical phenotypes

Current models of CDH are based on three key inter-related pathophysiologies; pulmonary hypertension, pulmonary hypoplasia, and cardiac dysfunction (65). However, the severity of each of these may be variable and disproportionate. Although severe cardiac dysfunction is associated with larger diaphragmatic defects, smaller lung volumes, and more severe pulmonary hypertension, it may also be observed in patients with smaller diaphragmatic defects and milder respiratory compromise (62, 63, 67). Improved recognition and characterization of individual clinical phenotypes may be

an important concept in CDH, and potentially inform more effective, targeted therapies.

Echocardiographic assessment of cardiac function and hemodynamics in congenital diaphragmatic hernia

The absence of standardized definitions and measurement tools are a major ongoing barrier to hemodynamic research and clinical management in CDH.

A variety of functional echocardiographic parameters have been used to assess cardiac function and pulmonary hypertension in CDH, as listed in **Tables 1**, **4**. However, each has practical and technical limitations and there is no single "gold standard" measure (72). Though useful definitions of cardiac dysfunction and pulmonary hypertension have been employed in individual studies, there is no established consensus agreement on these (58, 73).

To enable consistent multi-center hemodynamic data collection four actions are required:

1. Recommendations for routine use of functional echocardiography in acute CDH care
2. International consensus on measurement parameters, definitions and classification of cardiac function and pulmonary hypertension based on existing international guidance and adapted specifically for CDH (74–78).
3. Exploration of multi-modal measures of hemodynamic performance in CDH, including systemic oxygen delivery, cardiac output and microcirculatory function.

Bedside assessment of hemodynamic function in congenital diaphragmatic hernia

Echocardiographic assessment should be accompanied by wider multi-modal physiological assessment, as in any infant with hemodynamic instability. Where possible, near infrared spectroscopy enables monitoring of systemic oxygen delivery and may precede (79) changes in plasma lactate or end organ injury (80, 81). Exploratory studies are combining cerebral NIRS with EEG to investigate neuro-cardiovascular coupling (82).

Invasive arterial monitoring is a requisite during hemodynamic instability for monitoring of blood pressure and arterial gas sampling. However, line position and the impact of intra and extracardiac shunts must be considered. Right to left ductal shunting will decrease pH, PaO_2 and

TABLE 4 Investigations of biomarkers of cardiac function and pulmonary hypertension in CDH.

References	Population	Plasma biomarker	Relationship to hemodynamic performance	Available for routine clinical use
Partridge et al. (84)	132 CDH	BNP	BNP correlated with pulmonary hypertension and need for ECMO. No cardiac function data.	Y
Guslits et al. (85)	49 CDH	BNP levels at age 1–5 weeks	BNP level predicted adverse outcome at 3–5 weeks (ongoing respiratory support or death). No cardiac function data.	Y
Avitabile et al. (61)	220 CDH	BNP levels pre-repair, post-repair and recovery (>1 week post repair)	Increased BNP level associated with reduced strain in recovery, but not pre- or immediately post-op.	Y
Baptista et al. (86)	28 CDH	NT-proBNP in first 24 h of life	NT-proBNP correlated with RV MPI, TV E:A, and PAP.	Y
Snoek et al. (89)	128 CDH	High sensitivity troponin (hsTnT) and NT-proBNP on day 1	NT-proBNP and hsTNT did not predict death, PH, ECMO, or BPD. No cardiac function data.	Y
Heindel et al. (87)	44 CDH	NT-proBNP at 6, 12, 24, and 48 h of life	NT-proBNP correlated with qualitative cardiac dysfunction at 24 h, 48 h, and 7 days, and was higher in ECMO group.	Y
Bo et al. (69)	63 CDH	NT-proBNP measured daily for the first 7 days on ECMO	Significantly higher NT-proBNP values on days 3–7 in patients with ECMO weaning failure. Doubling in mortality in patients with increasing NT-proBNP on days 4–7.	Y
Gupta et al. (88)	2337 CDH	NT-proBNP recorded during neonatal admission	NT-proBNP correlated with cardiac dysfunction (RV or LV), mortality and larger defects.	Y
Keller et al. (155)	40 CDH	Endothelin 1 (ET-1) measured serially in first 2 weeks of life	ET-1 correlated with PH at 2 weeks of age. No cardiac function data.	N
Patel et al. (90)	10 CDH	VEGFA and placental growth factor (PLGF) measured serially during neonatal period	VEGFA:PLGF ratio correlated with RV diastolic function, PH and oxygenation index, and higher in non-survivors at days 3 and 14.	N
Kipfmueller et al. (91)	30 CDH	Soluble receptor for advanced glycation end products (sRAGE) at 6, 12, 24, 48 h and 7–10 days	sRAGE lower in CDH than controls and lower in ECMO cases. sRAGE correlated with pulmonary hypertension and fetal lung volume. No cardiac function data.	N

BNP, brain natriuretic peptide; NT-proBNP, N terminal proBNP; RV, right ventricle; MPI, myocardial performance index; TV, tricuspid valve; PAP, pulmonary artery pressure; PH, pulmonary hypertension; BPD, bronchopulmonary dysplasia.

increase $PaCO_2$ of post-ductal measurements, whereas a right-to-left atrial shunt, whilst uncommon in CDH, will also reduce pre-ductal oxygenation (71, 83).

Biomarkers of cardiac function in congenital diaphragmatic hernia

Plasma biomarkers may be useful in CDH to assess cardiac performance and pulmonary hypertension. These may be produced in response to hemodynamic compromise, or contribute to the primary pathways mediating CDH pathophysiology.

Natriuretic peptides brain natriuretic peptide (BNP) and its precursor N-terminal (NT) proBNP are established biomarkers in other pulmonary hypertensive diseases. BNP has a short half-life complicating measurement, but in CDH has been shown to be associated with pulmonary hypertension, need for ECMO, predictive of adverse outcome at 1 month of age, **Table 4** (84, 85). Recently Avitabile et al. also demonstrated that BNP was associated with impaired RV strain after CDH repair, though not in the pre-operative period (61).

NT-proBNP has a longer half-life making clinical measurement more reliable, and has been observed to correlate with cardiac dysfunction, pulmonary hypertension, and ECMO use in case series and large registry analysis (86–88). However, neither NT-proBNP, nor high sensitivity troponin (hsTnT) correlated with outcomes in a recent RCT of ventilation modalities in CDH (89).

Other potential biomarkers include the vascular endothelial growth factor-A (VEGFA) and placental growth factor (PLGF), the ratio of which correlated with RV diastolic dysfunction in a pilot investigation (90). Also, the soluble receptor of advanced glycation end products (sRAGE) is a new potential mediator of endothelial dysfunction in CDH, associated with mortality, severity of PH, and adverse outcome in CDH (91). MicroRNA's, a group of small non-coding RNA, are involved in the development and function of the lungs and the pulmonary vasculature in CDH, though to date their relationship to cardiac dysfunction in CDH remains unstudied (92–94).

The heart and the brain in congenital diaphragmatic hernia

Congenital diaphragmatic hernia is associated with neurodevelopmental impairment in a significant minority of affected people (95). Altered fetal brain development and postnatal brain injury may be contributing factors, and neuro-cardiovascular interactions may be central to these (82, 96).

Abnormalities in ventricular size and function may plausibly lead to alterations in cerebral blood flow, as is also suspected in infants with hypoplastic left heart syndrome (97).

This is itself a major topic for further discussion and investigation in CDH, beyond the scope of this review.

Management of cardiac dysfunction in congenital diaphragmatic hernia

Physiological approaches to managing the transition at birth

Recent animal and human feasibility studies have explored the use of physiologically based strategies for managing the transition at birth, combining lung recruitment with delayed cord clamping. These have demonstrated short-term improvements in pulmonary blood flow, pulmonary artery pressure and systemic blood pressure, though the impact on cardiac function *per se* has not been directly investigated (98–100). Randomized trials are in progress, but do not include cardiac function as a key outcome measure (101, 102).

Pharmacological therapies

Hemodynamic pharmacotherapy in CDH remains a challenging and unresolved issue. Historical approaches focused on maintaining systemic blood pressure and promoting pulmonary arterial vasodilation. The list of possible pharmacological therapies is ever-increasing, but with limited and often contradictory evidence leading to clinical confusion and risk of inappropriate use (103).

The current use of pulmonary vasodilators exemplifies this. Inhaled nitric oxide and sildenafil (in oral and intravenous formulations) are widely used in CDH patients with the intention of reducing pulmonary vascular resistance *via* endogenous nitric oxide pathways (104). However, only a minority of recipients demonstrate improved oxygenation, historic RCTs did not demonstrate improved outcomes, and recent registry analysis has suggested that iNO may be associated with increased mortality (105, 106).

Improved understanding of cardiac dysfunction may help address the uncertainty and anxiety around the use of these agents. The presence of LV dysfunction appears to be associated with non-response to iNO and sildenafil (107, 108). Possible mechanisms may be post-capillary hypertension secondary to LV dysfunction unresponsive to pre-capillary vasodilatation, or increased pulmonary blood flow exacerbating LV dysfunction (109).

The actions of other cardiovascular therapies which have been directly investigated in CDH are summarized in **Table 5**. Milrinone, a phospho-diesterase 3 inhibitor acting on the endogenous prostacyclin pathway appears well suited to treat both RV and LV dysfunction in CDH, for its inotropic, lusitropic

TABLE 5 Hemodynamic therapies investigated in CDH.

Therapy	Class	Presumed actions	Use in CDH (62, 124, 127)	Evidence in CDH
Inhaled nitric oxide	Nitric oxide analog	Pulmonary vasodilator	62–65%	Improved oxygenation in minority (30%) of unselected recipients. No improvement in outcome (79). Non-response may be linked to LV dysfunction (81).
Sildenafil (IV or enteral)	Phospho-diesterase 5 inhibitor	Pulmonary and systemic vasodilator	IV: 16% Any: 22%	Improved oxygenation in minority of recipients. Non-response associated with LV dysfunction (64). Ongoing CoDiNOS RCT of IV sildenafil vs. iNO in progress (73).
Milrinone	Prostacyclin analog	+ve inotrope and lusitrope. Pulmonary and systemic vasodilator	33–42%	Improved oxygenation and RV diastolic velocities (84). No effect on LV dimensions and atrial and ductal shunts (85). RCT in progress (57).
Vasopressin	Vasopressin analog	Pulmonary vasodilator, systemic vasoconstriction	Not known	Increased blood pressure, reduced systemic:pulmonary artery pressure ratio, improved oxygenation (89).
Levosimendan	Calcium sensitizer	+ve inotrope	Not known	Improved RV and LV function and reduced vasopressor-inotrope score (88)
Prostaglandin E1	Prostaglandin	Maintain ductal patency, pulmonary vasodilator	9–11%	Improved indices of PAP, LV function and oxygenation (120–122).
ECMO	–	Mechanical support	50%	Improved biventricular function on ECMO (69)

and pulmonary vasodilating effects (110). In a case series of infants with pre-existent RV dysfunction milrinone use was associated with improved oxygenation and RV diastolic function (111). However, a recent retrospective analysis in mild to moderate CDH observed no effect on oxygenation or LV dimensions (112). An RCT of early milrinone use in CDH is ongoing but does not include cardiac dysfunction as an enrollment parameter (113).

Levosimendan a calcium-sensitizing drug is commonly used in infants with congenital heart defects in the setting of low cardiac output syndrome (114). There is preliminary evidence that levosimendan is also associated with improvement of right and left ventricular dysfunction and a decrease in the Vasopressor-Inotropic Score in CDH (115).

Vasopressin use in CDH has also been associated with improved blood pressure, and reduced pulmonary:systemic blood pressure ratio, though the specific impact on LV function remains unstudied (116). The utility of systemic vasoconstrictors in CDH is unclear, and may depend on individual patient pathophysiology. In the setting of severe LV dysfunction increasing afterload may exacerbate ventricular failure (117). However, if LV function is preserved and RV function impaired there may be theoretical benefits to increasing systemic vascular resistance; to improve RV coronary blood flow, augment LV function *via* the eponymous Anrep effect, and restore septal positioning (51, 118, 119). Further investigation is required to understand which patient phenotypes are likely to benefit, and which of these potential mechanisms are beneficial in the clinical setting.

Prostaglandin E1 (PGE_1) use been proposed in early cardiovascular management in CDH to maintain ductal patency, as well as for its pulmonary vasodilating properties. In the setting of supra-systemic pulmonary hypertension a patent ductus permits right-to-left shunting, reducing the effective afterload on the RV, and supporting systemic blood flow, with theoretical benefits in the setting of both RV and/or LV dysfunction. Case series have demonstrated improvements in pulmonary artery pressure, oxygenation and LV function with PGE_1 use, however the optimal timing, dosing and duration remains undefined (120–122).

From the investigations described here it has become clear that there is no universally effective single agent for treating cardiac function and pulmonary hypertension in CDH. However, that does not necessarily mean that current therapies are ineffective. Instead new therapeutic *strategies* may be required, based on characterization of individual patient phenotype, including the relative contributions of RV and LV dysfunction, pre and postcapillary PVR, and ductal patency. This will allow investigation of the efficacy of targeted, pathophysiology-based therapeutic approaches, rather than indiscriminate use of single agents.

Hydrocortisone is also frequently used in the management of infants with CDH as an adjunct treatment for hypotensive cardiovascular compromise, and appears to elicit useful increases in both systemic vascular resistance and cardiac output (123, 124). Up to two thirds of CDH cases may have biochemical evidence of adrenal insufficiency in the immediate pre and post-operative periods, as observed by Kamath et al. (125). Low cortisol levels were associated with need for higher levels of cardio-respiratory support including ECMO, but not survival.

Extra-corporeal membrane oxygenation and cardiac function in congenital diaphragmatic hernia

Early ventricular dysfunction is predictive of extra-corporeal membrane oxygenation (ECMO) use in CDH,

and ECMO may be an important means of mechanically supporting the failing heart (62). However, there is minimal published data on longitudinal changes of ventricular function during the ECMO therapy. Additionally, decision-making regarding the relative benefits of venoarterial or venovenous ECMO based on the severity of concomitant ventricular dysfunction is at present supported only by clinician experience and opinion and not by published data (126). Bo et al. recently observed that ventricular function improves rapidly over the first days of life on ECMO support and may be monitored using biomarkers including NT-pro BNP (69).

Although cardiac dysfunction is commonly observed before and immediately after commencement of ECMO, it is rarely the cause of failure to wean support. As discussed above, severe LV dysfunction is a transient phenomenon and typically resolves in a matter of days. Nevertheless, ongoing right ventricular dysfunction after ECMO has been described, associated with persistent elevation of pulmonary artery pressure (61).

The two principal phenotypes responsible for ECMO weaning failure are first severe ventilation failure secondary to pulmonary hypoplasia, and second severe ongoing pulmonary hypertension. The latter may be due to irreversible developmental anomalies of pulmonary vasculature structure and function, decruitment of lung, or other factors such as ongoing infection.

Improved understanding of the relative contribution of cardiac dysfunction to individual pathophysiology before and during ECMO may be important for improved ECMO management strategies and help resolve ongoing uncertainties and controversies (127, 128).

Future priorities in congenital diaphragmatic hernia cardiac function therapy

To achieve effective use of these and other cardiovascular therapies in CDH future priorities should include:

- Standardized hemodynamic assessment parameters in CDH, incorporating routine functional echocardiography. Consensus definitions of pulmonary hypertension and cardiac function and dysfunction based on standardized measures.
- Pathophysiology-based treatment strategies rather than "one-size fits all" approach to use of single agents (1).
- Routine assessment of cardiac function in studies of hemodynamic therapies, as in the recent CoDiNOS trial of IV sildenafil (73).
- Investigation of the relationship between LV performance and response to pulmonary vasodilator therapies.
- Improved understanding of cardiac function to guide individualized ECMO strategies including improved patient selection and timing of repair.
- Investigation of whether improvement in cardiac function translates to improved short and long-term clinical outcomes.

Surgical repair and cardiac function in congenital diaphragmatic hernia

Current international guidelines recommend delaying CDH repair until physiological stability including normalized blood pressure and lactate have been achieved, but do not specifically reference cardiac function (129). RV function may deteriorate within 72 h of surgical repair (49). In a recent cohort Avitabile et al. observed that RV strain improved in the recovery phase after surgery, though over 50% of cases had ongoing reduction in RV strain (61). Chronic elevation of PVR, exacerbated by surgery, may contribute to this ongoing RV dysfunction. Conversely in the LV, Tanaka et al. have observed improved LV diastolic wall strain following early CDH repair, potentially as a result of removing the compressive action of the hernia (130). Further investigation is required to fully understand the impacts of the herniating abdominal contents, anesthesia and the surgical techniques on cardiac function and hemodynamics in CDH.

Cardiac loading conditions may also be altered in the post-operative period. Chylothorax occurs in 5% of CDH cases after repair or ECMO cannulation, possibly resulting from superior vena cava obstruction (131, 132). The associated reduction in preload may in turn affect ventricular function and cardiac output.

Long-term cardiac function in congenital diaphragmatic hernia

The natural history of long-term cardiac function in CDH is not well understood.

Kraemer et al. observed that pulmonary hypertension largely resolves in childhood in CDH survivors (133). Similarly, a recent literature review identified highly variable rates of pulmonary hypertension (4.5–38%) in CDH survivors over 2 years old, and diminishing rates by 5 years of age (134). However, the limitations of echocardiographic techniques make accurate non-invasive assessment of pulmonary artery pressure challenging. Ventricular function may be a more sensitive measure of ongoing changes in RV loading conditions and the systemic circulation, as

TABLE 6 The heart in CDH: Knowns, unknowns and future research priorities.

Fetus	Early postnatal period	Peri-operative period and ECMO	Post discharge, childhood and beyond
Knowns			
Fetal cardiac hypoplasia: Preliminary evidence of increase in ratio of right:left sided dimensions in later pregnancy in left-sided CDH.	Potential for RV and/or LV dysfunction in transitional period.	Improvement of cardiac function during ECMO	Preliminary evidence of cardiac dysfunction at discharge and into childhood
	Relationship between early ventricular dysfunction and neonatal outcome	Preliminary evidence of patterns of RV and LV function post op	
Unknowns/future priorities			
Mechanisms of ventricular hypoplasia	Mechanisms of postnatal cardiac dysfunction	Contribution of cardiac dysfunction to pathophysiology pre-and during ECMO	Natural history and clinical significance on long-term cardiac dysfunction in CDH survivors
Pre-natal cardiac function	Effect of cardio-tropes on outcome in CDH	Utility of cardiac function assessment to guide therapeutic strategy including timing of CDH repair and ECMO	
Relationship between pre-natal cardiac function, cardiac dimensions	Relationship between pulmonary vasodilators and LV function	Post-natal patterns of RV and LV function and contribution to post-operative morbidity.	
Predictive potential of fetal cardiac dimensions and function	Impact of pathophysiology-based transitional management on cardiac function	Impact of related morbidities on cardiac function including nutrition, infection, gastro-esophageal reflux.	Long-term ventriculo-arterial interactions in CDH survivors
Relationship between fetal cardiac dimensions and function and postnatal cardiac dimensions and function			
Effect of fetal therapies on pre- and post- natal cardiac development and function			
International consensus, standardized assessment tools and definitions of cardiac function and pulmonary hypertension for research and clinical management			
Relationship between cardiac development and function, fetal brain development, postnatal brain injury, and long-term neurological function			
Phenotyping of CDH pathophysiology: relative contributions and frequency of cardiac dysfunction, pulmonary hypertension and pulmonary hypoplasia			
Cellular, genetic and metabolic factors associated with myocardial dysfunction at all ages			

demonstrated in non-CDH pulmonary hypertensive diseases (135–137).

Analysis in our own center demonstrated abnormal RV and LV strain in 70% and 44% of CDH patients respectively at the time of neonatal discharge (Massolo et al., unpublished data). Furthermore, Egan et al. observed abnormal RV strain in CDH survivors at a median of age of 6 years (138). Altered cardiac function, in conjunction with ongoing respiratory compromise, may conceivably contribute to functional exercise restriction in a minority of CDH patients (139).

In survivors of preterm birth there is increasing evidence that altered ventriculo-arterial interactions may contribute to adult cardiovascular dysfunction, raising potential concerns that similar pathologies might occur in CDH (140–142). Prospective, longitudinal studies are now required to understand lifelong patterns and functional significance of cardiac function in CDH.

Cardiac catheterization may be an important adjunct for longitudinal hemodynamic assessment in CDH. Limited case series have demonstrated the ability to directly assess pulmonary artery and intra-cardiac pressures, including in the left heart, ventricular outputs, occult shunts, additional congenital lesions, and responsiveness to therapies including pulmonary vasodilators in CDH (143, 144). Earlier, standardized use of cardiac catheterization in cases of CDH with ongoing hemodynamic compromise, is an important future consideration.

In young adults who have been born prematurely and have low adaptive capacity during exercise cardiac catheterization has revealed evidence of pulmonary hypertension (145). This raises the question of whether catheterization may also be indicated in CDH survivors who have ongoing evidence of cardio-respiratory exercise intolerance (146, 147).

Discussion

There is increasing evidence and recognition of the heart's contribution to disease pathophysiology in CDH (68). However, the "knowns" are outweighed by ongoing uncertainties or "unknowns," as summarized in **Table 6**. Addressing these may be critical to developing new therapeutic approaches and improved outcomes.

Beginning with the fetus, further investigation is required to investigate the nature and mechanisms of fetal LV hypoplasia at a cellular, metabolic, genetic and morphometric level, as well as the functional significance for fetal hemodynamics including cerebral perfusion. The relationship between fetal cardiac dimensions and function, early postnatal function

and outcomes is also a priority that may identify improved prenatal predictors and postnatal therapeutic strategies. Assessment of any fetal therapies should include the impact on the heart, as well as the lungs and pulmonary vasculature.

Though RV and LV function have been characterized in early postnatal life, a deeper physiological understanding of underlying ventricular interdependence, ventriculo-arterial and cardio-respiratory interactions at this time will enable more informed, physiology-based therapeutic approaches.

Use of cardiovascular pharmacotherapies in CDH remains a challenging area. Ongoing trials of milrinone and pulmonary vasodilators may shed some light (73, 113). Hemodynamic assessment should be incorporated in all future interventional studies in CDH. To do so requires urgent consensus on what to measure, how, and when, as well as standardized definitions of cardiac function and pulmonary hypertension. This will enable the robust, multi-center data collection and analysis required in this rare disease.

Investigating new therapeutic paradigms may also be important. Rather than a "one size fits all" approach, improved assessment of individual patient pathophysiological phenotype and the relative contributions of cardiac function, alongside pulmonary hypertension and ventilatory function, may lead to improved, targeted use of therapies including pulmonary vasodilators, cardiotropes, and vasopressors. This approach may also improve ECMO strategies addressing thorny uncertainties around patient selection and timing of repair both on and off ECMO.

The relationship between the heart and the brain in CDH is also a critical area for research, to understand the neuro-cardiovascular coupling mechanisms contributing to fetal brain development and postnatal injury in CDH, and their relationship to long-term neurodevelopment.

Finally, the long-term nature of cardiovascular function in CDH survivors is another priority, to determine whether longer-term changes in cardiac morphology and function, or abnormalities of the systemic or pulmonary circulation, impact functional status later in life.

Addressing each of these uncertainties will require innovative approaches. First, in terms of methodology combining human clinical investigation, animal studies, and novel cellular and organoid models, as well as new imaging modalities including advanced functional echocardiography and MR (148–150). Second, by applying learning from other related conditions, including congenital heart disease and non-CDH pulmonary hypertensive disease. Third, and above all, collaboration is required to share knowledge, consolidate expertise and capabilities between researchers, clinicians and people with CDH themselves.

We hope that this review can act as a call to action, highlighting the importance of the heart and the next steps to progress our understanding, develop new therapies, and improve outcomes in CDH.

Author contributions

NP, AM, UK, and FK contributed to the concept, outline of the manuscript, reviewed drafts, and approved the final manuscript. NP provided co-ordination of each authors' contributions. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Patel N, Massolo AC, Kipfmueller F. Congenital diaphragmatic hernia-associated cardiac dysfunction. *Semin Perinatol.* (2020) 44:151168. doi: 10.1053/j.semperi.2019.07.007

2. Montalva L, Lauriti G, Zani A. Congenital heart disease associated with congenital diaphragmatic hernia: a systematic review on incidence, prenatal diagnosis, management, and outcome. *J Pediatr Surg.* (2019) 54:909–19. doi: 10.1016/j.jpedsurg.2019.01.018

3. Fraser CD, Hill KD, Wallace A, Chiswell K, Zhou X, Jelin EB, et al. The prevalence and impact of congenital diaphragmatic hernia among patients undergoing surgery for congenital heart disease. *Semin Thorac Cardiovasc Surg.* (2019) 31:69–77. doi: 10.1053/j.semtcvs.2018.09.014

4. Graziano JN. Cardiac anomalies in patients with congenital diaphragmatic hernia and their prognosis: a report from the congenital diaphragmatic hernia study group. *J Pediatr Surg.* (2005) 40:1045–9. doi: 10.1016/j.jpedsurg.2005.03.025

5. Kardon G, Ackerman KG, McCulley DJ, Shen Y, Wynn J, Shang L, et al. Congenital diaphragmatic hernias: from genes to mechanisms to therapies. *Dis Model Mech.* (2017) 10:955–70. doi: 10.1242/dmm.028365

6. Losty PD, Connell MG, Freese R, Laval S, Okoye BO, Smith A, et al. Cardiovascular malformations in experimental congenital diaphragmatic hernia. *J Pediatr Surg.* (1999) 34:1352–8. doi: 10.1016/S0022-3468(99)90152-5

7. Yu L, Hernan RR, Wynn J, Chung WK. The influence of genetics in congenital diaphragmatic hernia. *Semin Perinatol.* (2020) 44:151169. doi: 10.1053/j.semperi.2019.07.008

8. Brosens E, Peters NCJ, van Weelden KS, Bendixen C, Brouwer RWW, Sleutels F, et al. Unraveling the genetics of congenital diaphragmatic hernia: an ongoing challenge. *Front Pediatr.* (2022) 9:800915. doi: 10.3389/fped.2021.800915

9. Gray BW, Fifer CG, Hirsch JC, Tochman SW, Drongowski RA, Mychaliska GB, et al. Contemporary outcomes in infants with congenital heart disease and bochdalek diaphragmatic hernia. *Ann Thorac Surg.* (2013) 95:929–34. doi: 10.1016/j.athoracsur.2012.07.010

10. Balks J, Mueller A, Herberg U, Geipel A, Gembruch U, Schroeder L, et al. Therapy and outcome of neonates with congenital diaphragmatic hernia and congenital heart defects. *Z Geburtshilfe Neonatol.* (2021) 225:432–40. doi: 10.1055/a-1392-1460

11. Balduf K, Kumar TKS, Boston U, Sathanandam S, Lee MV, Jancelewicz T, et al. Improved outcomes in management of hypoplastic left heart syndrome associated with congenital diaphragmatic hernia: an algorithmic approach. *Semin Thorac Cardiovasc Surg.* (2018) 30:191–6. doi: 10.1053/j.semtcvs.2018.02.010

12. Losty PD, Vanamo K, Rintala RJ, Donahoe PK, Schnitzer JJ, Lloyd DA. Congenital diaphragmatic hernia – Does the side of the defect influence the incidence of associated malformations? *J Pediatr Surg.* (1998) 33:507–10. doi: 10.1016/S0022-3468(98)90099-9

13. Siebert JR, Haas JE, Beckwith JB. Left ventricular hypoplasia in congenital diaphragmatic hernia. *J Pediatr Surg.* (1984) 19:567–71. doi: 10.1016/S0022-3468(84)80105-0

14. Byrne FA, Keller RL, Meadows J, Miniati D, Brook MM, Silverman NH, et al. Severe left diaphragmatic hernia limits size of fetal left heart more than does right diaphragmatic hernia. *Ultrasound Obstet Gynecol.* (2015) 46:688–94. doi: 10.1002/uog.14790

15. Vogel M, McElhinney DB, Marcus E, Morash D, Jennings RW, Tworetzky W. Significance and outcome of left heart hypoplasia in fetal congenital diaphragmatic hernia. *Ultrasound Obstet Gynecol.* (2010) 35:310–7. doi: 10.1002/uog.7497

16. Baumgart S, Paul JJ, Huhta JC, Katz AL, Paul KE, Spettell C, et al. Cardiac malposition, redistribution of fetal cardiac output, and left heart hypoplasia reduce survival in neonates with congenital diaphragmatic hernia requiring extracorporeal membrane oxygenation. *J Pediatr.* (1998) 133:57–62. doi: 10.1016/s0022-3476(98)70178-7

17. VanderWall KJ, Kohl T, Adzick NS, Silverman NH, Hoffman JI, Harrison MR. Fetal diaphragmatic hernia: echocardiography and clinical outcome. *J Pediatr Surg.* (1997) 32:223–5. doi: 10.1016/S0022-3468(97)90183-4

18. Massolo AC, Romiti A, Viggiano M, Vassallo C, Ledingham MA, Lanzone A, et al. Fetal cardiac dimensions in congenital diaphragmatic hernia: relationship with gestational age and postnatal outcomes. *J Perinatol.* (2021) 41:1651–9. doi: 10.1038/s41372-021-00986-y

19. Thébaud B, Azancot A, de Lagausie P, Vuillard E, Ferkadji L, Benali K, et al. Congenital diaphragmatic hernia: antenatal prognostic factors. Does cardiac ventricular disproportion in utero predict outcome and pulmonary hypoplasia? *Intensive Care Med.* (1997) 23:1062–9. doi: 10.1007/s001340050457

20. Yamoto M, Inamura N, Terui K, Nagata K, Kanamori Y, Hayakawa M, et al. Echocardiographic predictors of poor prognosis in congenital diaphragmatic hernia. *J Pediatr Surg.* (2016) 51:1926–30. doi: 10.1016/j.jpedsurg.2016.09.014

21. Dekoninck P, Richter J, van Mieghem T, van Schoubroeck D, Allegaert K, de Catte L, et al. Cardiac assessment in fetuses with right-sided congenital diaphragmatic hernia: case-control study. *Ultrasound Obstet Gynecol.* (2014) 43:432–6. doi: 10.1002/uog.12561

22. Mardy C, Blumenfeld YJ, Arunamata AA, Girsen AI, Sylvester KG, Halabi S, et al. In fetuses with congenital lung masses, decreased ventricular and atrioventricular valve dimensions are associated with lesion size and clinical outcome. *Prenat Diagn.* (2020) 40:206–15. doi: 10.1002/pd.5612

23. Bravo-Valenzuela NJM, Peixoto AB, Araujo Júnior E. Prenatal diagnosis of total anomalous pulmonary venous connection: 2D and 3D echocardiographic findings. *J Clin Ultrasound.* (2021) 49:240–7. doi: 10.1002/jcu.22973

24. Stressig R, Fimmers R, Eising K, Gembruch U, Kohl T. Preferential streaming of the ductus venosus and inferior caval vein towards the right heart is associated with left heart underdevelopment in human fetuses with left-sided diaphragmatic hernia. *Heart.* (2010) 96:1564–8. doi: 10.1136/hrt.2010.196550

25. Degenhardt J, Enzensberger C, Tenzer A, Kaweckir A, Kohl T, Widriani E, et al. Myocardial function pre- and post-fetal endoscopic tracheal occlusion (FETO) in fetuses with left-sided moderate to severe congenital diaphragmatic hernia. *Ultraschall Med.* (2017) 38:65–70. doi: 10.1055/s-0041-108501

26. Dekoninck P, Jan D, Mieghem T, Richter J, Deprest J. Speckle tracking echocardiography in fetuses diagnosed with congenital diaphragmatic hernia. *Prenat Diagn.* (2014) 34:1262–7.

27. van Mieghem T, Gucciardo L, Doné E, van Schoubroeck D, Graatsma EM, Visser GHA, et al. Left ventricular cardiac function in fetuses with congenital diaphragmatic hernia and the effect of fetal endoscopic tracheal occlusion. *Ultrasound Obstet Gynecol.* (2009) 34:424–9. doi: 10.1002/uog.7340

28. Lemini MC, Josep M, Montiel JG. Characterizing cardiac dysfunction in fetuses with left congenital diaphragmatic hernia. *Prenat Diagn.* (2018) 38:422–7. doi: 10.1002/pd.5253

29. Kaya B, Tayyar A, Sezer S, Kaya S. The assessment of cardiac function with tissue Doppler imaging in fetuses with congenital diaphragmatic hernia. *J Mater Fetal Neonatal Med.* (2020) 33:1233–8. doi: 10.1080/14767058.2019.1674806

30. Coffman ZJ, McGahren ED, Vergales BD, Saunders CH, Vergales JE. The effect of congenital diaphragmatic hernia on the development of left-sided heart structures. *Cardiol Young.* (2019) 29:813–8. doi: 10.1017/S1047951119000891

31. Kailin JA, Dhillon GS, Maskatia SA, Cass DL, Shamshirsaz AA, Mehollin-ray AR, et al. Fetal left-sided cardiac structural dimensions in left-sided congenital diaphragmatic hernia – association with severity and impact on postnatal outcomes. *Prenat Diagn.* (2017) 37:502–9. doi: 10.1002/pd.5045

32. Yamoto M, Tanaka Y, Fukumoto K, Miyake H, Nakajima H, Koyama M, et al. Cardiac fetal ultrasonographic parameters for predicting outcomes of isolated left-sided congenital diaphragmatic hernia. *J Pediatr Surg.* (2015) 50:2019–24. doi: 10.1016/j.jpedsurg.2015.08.016

33. Schwartz SM, Vermilion RP, Hirschl RB. Evaluation of left ventricular mass in children with left-sided congenital diaphragmatic hernia. *J Pediatr.* (1994) 125:447–51. doi: 10.1016/s0022-3476(05)83293-7

34. Teramoto H, Puri P. Gene expression of insulin-like growth factor-1 and epidermal growth factor is downregulated in the heart of rats with nitrofen-induced diaphragmatic hernia. *Pediatr Surg Int.* (2001) 17:284–7. doi: 10.1007/s003830100595

35. Guarino N, Shima H, Puri P. The hypoplastic heart in congenital diaphragmatic hernia: reduced expression of basic fibroblast growth factor and platelet-derived growth factor. *Pediatr Surg Int.* (2000) 16:243–6. doi: 10.1007/s003830050737

36. Zhaorigetu S, Gupta VS, Jin D, Harting MT. Cardiac energy metabolism may play a fundamental role in congenital diaphragmatic hernia-associated ventricular dysfunction. *J Mol Cell Cardiol.* (2021) 157:14–6. doi: 10.1016/j.yjmcc.2021.04.001

37. Pelizzo G, Bussani R, Zandonà L, Custrin A, Bellieni CV, de Silvestri A, et al. Cardiac adaptation to severe congenital diaphragmatic hernia. *Fetal Pediatr Pathol.* (2016) 35:10–20. doi: 10.3109/15513815.2015.1122125

38. Karamanoukian HL, Glick PL, Wilcox DT, O'Toole SJ, Rossman JE, Azizkhan RG. Pathophysiology of congenital diaphragmatic hernia XI: anatomic and biochemical characterization of the heart in the fetal lamb CDH model. *J Pediatr Surg.* (1995) 30:925–8. doi: 10.1016/0022-3468(95)90314-3

39. Tannuri U. Heart hypoplasia in an animal model of congenital diaphragmatic hernia. *Rev Hosp Clin.* (2001) 56:173–8. doi: 10.1590/s0041-87812001000600003

40. Grossfeld P, Nie S, Lin L, Wang L, Anderson RH. Hypoplastic left heart syndrome: a new paradigm for an old disease? *J Cardiovasc Dev Dis.* (2019) 6:10. doi: 10.3390/jcdd6010010

41. Deprest JA, Nicolaides KH, Benachi A, Gratacos E, Ryan G, Persico N, et al. Randomized trial of fetal surgery for severe left diaphragmatic hernia. *N Engl J Med.* (2021) 385:107–18. doi: 10.1056/NEJMoa2027030

42. Marulanda K, Tsihlis ND, McLean SE, Kibbe MR. Emerging antenatal therapies for congenital diaphragmatic hernia-induced pulmonary hypertension in preclinical models. *Pediatr Res.* (2021) 89:1641–9. doi: 10.1038/s41390-020-01191-x

43. Luong C, Rey-Perra J, Vadivel A, Gilmour G, Sauve Y, Koonen D, et al. Antenatal sildenafil treatment attenuates pulmonary hypertension in experimental congenital diaphragmatic hernia. *Circulation.* (2011) 123:2120–31. doi: 10.1161/CIRCULATIONAHA.108.845909

44. Russo FM, Toelen J, Eastwood MP, Jimenez J, Miyague AH, Velde G. Transplacental sildenafil rescues lung abnormalities in the rabbit model of diaphragmatic hernia. *Thorax.* (2016) 71:517–25. doi: 10.1136/thoraxjnl-2015-207949

45. Kashyap AJ, Dekoninck PLJ, Rodgers KA, Thio M, Mcgillick EV, Amberg BJ, et al. Antenatal sildenafil treatment improves neonatal pulmonary hemodynamics and gas exchange in lambs with diaphragmatic hernia. *Ultrasound Obstet Gynecol.* (2019) 54:506–16. doi: 10.1002/uog.20415

46. Smith GCS. The STRIDER trial: one step forward, one step back. *Lancet Child Adolesc Health.* (2018) 2:80–1. doi: 10.1016/S2352-4642(17)30176-1

47. Lee FT, Marini D, Seed M, Sun L. Maternal hyperoxygenation in congenital heart disease. *Transl Pediatr.* (2021) 10:2197–209

48. Enzensberger C, Axt-Fliedner R, Degenhardt J, Kawecki A, Tenzer A, Kohl T, et al. Pulmonary vasoreactivity to materno-fetal hyperoxygenation testing in fetuses with hypoplastic left heart. *Ultraschall Med.* (2016) 37:195–200. doi: 10.1055/s-0034-1385668

49. Moenkemeyer F, Patel N. Right ventricular diastolic function measured by tissue doppler imaging predicts early outcome in congenital diaphragmatic hernia. *Pediatr Critic Care Med.* (2014) 15:49–55. doi: 10.1097/PCC.0b013e31829b1e7a

50. Harting MT. Congenital diaphragmatic hernia-associated pulmonary hypertension. *Semin Pediatr Surg.* (2017) 26:147–53. doi: 10.1053/j.sempedsurg.2017.04.008

51. Brooks H, Kirk ES, Vokonas PS, Urschel CW, Sonnenblick EH. Performance of the right ventricle under stress: relation to right coronary flow. *J Clin Invest.* (1971) 50:2176–83. doi: 10.1172/JCI106712

52. Aggarwal S, Stockmann P, Klein MD, Natarajan G. Echocardiographic measures of ventricular function and pulmonary artery size: prognostic markers of congenital diaphragmatic hernia? *J Perinatol.* (2011) 31:561–6. doi: 10.1038/jp.2011.3

53. Patel N, Mills JF, Cheung MMH. Assessment of right ventricular function using tissue doppler imaging in infants with pulmonary hypertension. *Neonatology.* (2009) 96:193–9. doi: 10.1159/000215585

54. Patel N, Mills JF, Cheung MMH. Use of the myocardial performance index to assess right ventricular function in infants with pulmonary hypertension. *Pediatr Cardiol.* (2009) 30:133–7. doi: 10.1007/s00246-008-9285-1

55. Altit G, Bhombal S, van Meurs K, Tacy TA. Diminished cardiac performance and left ventricular dimensions in neonates with congenital diaphragmatic hernia. *Pediatr Cardiol.* (2018) 39:993–1000. doi: 10.1007/s00246-018-1850-7

56. Nagiub M, Klein J, Gullquist S. Echocardiography derived pulmonary artery capacitance and right ventricular outflow velocity time integral on first day of life can predict survival in congenital diaphragmatic hernia. *Prog Pediatr Cardiol.* (2018) 48:107–10. doi: 10.1016/j.ppedcard.2018.01.001

57. Ferguson DM, Gupta VS, Lally PA, Luco M, Tsao K, Lally KP, et al. Early, postnatal pulmonary hypertension severity predicts inpatient outcomes in congenital diaphragmatic hernia. *Neonatology.* (2021) 118:147–54. doi: 10.1159/000512966

58. Lusk LA, Wai KC, Moon-Grady AJ, Steurer MA, Keller RL. Persistence of pulmonary hypertension by echocardiography predicts short-term outcomes in congenital diaphragmatic hernia. *J Pediatr.* (2015) 166:251–6. doi: 10.1016/j.jpeds.2014.10.024

59. Aggarwal S, Stockman PT, Klein MD, Natarajan G. The right ventricular systolic to diastolic duration ratio: a simple prognostic marker in congenital diaphragmatic hernia? *Acta Paediatr.* (2011) 100:1315–8. doi: 10.1111/j.1651-2227.2011.02302.x

60. Altit G, Bhombal S, van Meurs K, Tacy TA. Ventricular performance is associated with need for extracorporeal membrane oxygenation in newborns with congenital diaphragmatic hernia. *J Pediatr.* (2017) 191:28–34.e1. doi: 10.1016/j.jpeds.2017.08.060

61. Avitabile CM, Wang Y, Zhang X, Griffis H, Saavedra S, Adams S, et al. Right ventricular strain, brain natriuretic peptide, and mortality in congenital diaphragmatic hernia. *Ann Am Thorac Soc.* (2020) 17:1431–9. doi: 10.1513/AnnalsATS.201910-767OC

62. Patel N, Lally PA, Kipfmueller F, Massolo AC, Luco M, van Meurs KP, et al. Ventricular dysfunction is a critical determinant of mortality in congenital diaphragmatic hernia. *Am J Respir Crit Care Med.* (2019) 200:1522–30. doi: 10.1164/rccm.201904-0731OC

63. Patel N, Massolo AC, Paria A, Stenhouse EJ, Hunter L, Finlay E, et al. Early postnatal ventricular dysfunction is associated with disease severity in patients with congenital diaphragmatic hernia. *J Pediatr.* (2018) 203:400–7. doi: 10.1016/j.jpeds.2018.07.062

64. Massolo AC, Paria A, Hunter L, Finlay E, Davis CF, Patel N. Ventricular dysfunction, interdependence, and mechanical dispersion in newborn infants with congenital diaphragmatic hernia. *Neonatology.* (2019) 116:68–75. doi: 10.1159/000499347

65. Kinsella J, Steinhorn R, Mullen M, Hopper R, Keller R, Ivy D, et al. The left ventricle in congenital diaphragmatic hernia: implications for the management of pulmonary hypertension. *J Pediatr.* (2018) 197:17–22. doi: 10.1016/j.jpeds.2018.02.040

66. Gaffar S, Ellini AR, Ahmad I, Chen Y, Ashrafi AH. Left ventricular cardiac output is a reliable predictor of extracorporeal life support in neonates with congenital diaphragmatic hernia. *J Perinatol.* (2019) 39:648–53. doi: 10.1038/s41372-019-0348-3

67. Duy D, Patel N, Harting MT, Lally KP, Lally PA, Buchmiller TL. Early left ventricular dysfunction and severe pulmonary hypertension predict adverse outcomes in "low-risk" congenital diaphragmatic hernia. *Pediatr Crit Care Med.* (2020) 21:637–46. doi: 10.1097/PCC.0000000000002318

68. Patel N, Kipfmueller F. Cardiac dysfunction in congenital diaphragmatic hernia: pathophysiology, clinical assessment, and management. *Semin Pediatr Surg.* (2017) 26:154–8. doi: 10.1053/j.sempedsurg.2017.04.001

69. Bo B, Balks J, Gries K, Holdenrieder S, Mueller A, Kipfmueller F. Increased N-terminal Pro-B-Type natriuretic peptide during extracorporeal life support is associated with poor outcome in neonates with congenital diaphragmatic hernia. *J Pediatr.* (2021) 241:83–9. doi: 10.1016/j.jpeds.2021.09.034

70. Rosenkranz S, Gibbs JSR, Wachter R, de Marco T, Vonk-Noordegraaf A, Vachiéry JL. Left ventricular heart failure and pulmonary hypertension. *Eur Heart J.* (2016) 37:942–54. doi: 10.1093/eurheartj/ehv512

71. Wehrmann M, Patel SS, Haxel C, Cassidy C, Howley L, Cuneo B, et al. Implications of atrial-level shunting by echocardiography in newborns with congenital diaphragmatic hernia. *J Pediatr.* (2020) 219:43–7. doi: 10.1016/j.jpeds.2019.12.037

72. Rudski LG, Afilalo J. The blind men of indostan and the elephant in the echo lab. *J Am Soc Echocardiogr.* (2012) 25:714–7. doi: 10.1016/j.echo.2012.05.022

73. Cochius-Den Otter S, Schaible T, Greenough A, van Heijst A, Patel N, Allegaert K, et al. The CoDiNOS trial protocol: an international randomised controlled trial of intravenous sildenafil versus inhaled nitric oxide for the treatment of pulmonary hypertension in neonates with congenital diaphragmatic hernia. *BMJ Open.* (2019) 9:e032122. doi: 10.1136/bmjopen-2019-032122

74. Rudski LG, Lai WW, Afilalo J, Hua L, Handschumacher MD, Chandrasekaran K, et al. Guidelines for the echocardiographic assessment of the right heart in adults: a report from the american society of echocardiography. *J Am Soc Echocardiogr.* (2010) 23:685–713. doi: 10.1016/j.echo.2010.05.010

75. Tissot C, Singh Y, Sekarski N. Echocardiographic evaluation of ventricular function-for the neonatologist and pediatric intensivist. *Front Pediatr.* (2018) 6:79. doi: 10.3389/fped.2018.00079

76. de Boode WP, Singh Y, Molnar Z, Schubert U, Savoia M, Sehgal A, et al. Application of neonatologist performed echocardiography in the assessment and management of persistent pulmonary hypertension of the newborn. *Pediatr Res.* (2018) 84:68–77. doi: 10.1038/s41390-018-0082-0

77. Hansmann G, Koestenberger M, Alastalo TP, Apitz C, Austin ED, Bonnet D, et al. 2019 updated consensus statement on the diagnosis and treatment of pediatric pulmonary hypertension: the european pediatric pulmonary vascular disease network (EPPVDN), endorsed by AEPC, ESPR and ISHLT. *J Heart Lung Transplant.* (2019) 38:879–901. doi: 10.1016/j.healun.2019.06.022

78. Abman SH, Hansmann G, Archer SL, Ivy DD, Adatia I, Chung WK, et al. Pediatric pulmonary hypertension. *Circulation.* (2015) 132:2037–99. doi: 10.1161/CIR.0000000000000329

79. Dempsey EM, El-Khuffash AF. Objective cardiovascular assessment in the neonatal intensive care unit. *Arch Dis Child Fetal Neonatal Ed.* (2018) 103:F72–7. doi: 10.1136/archdischild-2017-313837

80. Lau PE, Cruz S, Garcia-Prats J, Cuevas M, Rhee C, Cass DL, et al. Use of renal near-infrared spectroscopy measurements in congenital diaphragmatic hernia patients on ECMO. *J Pediatr Surg.* (2017) 52:689–92. doi: 10.1016/j.jpedsurg.2017.01.015

81. Ryan A, Gilhooley M, Patel N, Reynolds BC. Prevalence of acute kidney injury in neonates with congenital diaphragmatic hernia. *Neonatology.* (2020) 117:88–94. doi: 10.1159/000503293

82. Hendrikx D, Costerus SA, Katrin Z, Perez-Ortiz A, Caicedo Dorado A, van Huffel S, et al. Neurocardiovascular coupling in congenital diaphragmatic hernia patients undergoing different types of surgical treatment. *Eur J Anaesthesiol.* (2021) 39:662–72. doi: 10.1097/EJA.0000000000001642

83. Gien J, Kinsella JP. Differences in preductal and postductal arterial blood gas measurements in infants with severe congenital diaphragmatic hernia. *Arch Dis Child Fetal Neonatal Ed.* (2016) 101:F314–8. doi: 10.1136/archdischild-2014-307714

84. Partridge EA, Hanna BD, Rintoul NE, Herkert L, Flake AW, Adzick NS, et al. Brain-type natriuretic peptide levels correlate with pulmonary hypertension and requirement for extracorporeal membrane oxygenation in congenital

diaphragmatic hernia. *J Pediatr Surg.* (2015) 50:263–6. doi: 10.1016/j.jpedsurg.2014.11.009

85. Guslits E, Steurer MA, Nawaytou H, Keller RL. Longitudinal B-Type natriuretic peptide levels predict outcome in infants with congenital diaphragmatic hernia. *J Pediatr.* (2021) 229:191–8. doi: 10.1016/j.jpeds.2020.09.064

86. Baptista MJ, Rocha G, Clemente F, Azevedo LF, Tibboel D, Leite-Moreira AF, et al. N-terminal-pro-B type natriuretic peptide as a useful tool to evaluate pulmonary hypertension and cardiac function in CDH infants. *Neonatology.* (2008) 94:22–30. doi: 10.1159/000112641

87. Heindel K, Holdenrieder S, Patel N, Bartmann P, Schroeder L, Berg C, et al. Early postnatal changes of circulating N-terminal-pro-B-type natriuretic peptide in neonates with congenital diaphragmatic hernia. *Early Hum Dev.* (2020) 146:105049. doi: 10.1016/j.earlhumdev.2020.105049

88. Gupta VS, Patel N, Kipfmueller F, Lally PA, Lally KP, Harting MT. Elevated proBNP levels are associated with disease severity, cardiac dysfunction, and mortality in congenital diaphragmatic hernia. *J Pediatr Surg.* (2021) 56:1214–9. doi: 10.1016/j.jpedsurg.2021.02.042

89. Snoek KG, Kraemer US, Ten Kate CA, Greenough A, van Heijst A, Capolupo I, et al. High-Sensitivity troponin T and N-terminal pro-brain natriuretic peptide in prediction of outcome in congenital diaphragmatic hernia: results from a multicenter, randomized controlled trial. *J Pediatr.* (2016) 173:245–9. doi: 10.1016/j.jpeds.2016.03.026

90. Patel N, Moenkemeyer F, Germano S, Cheung MMH. Plasma vascular endothelial growth factor A and placental growth factor: novel biomarkers of pulmonary hypertension in congenital diaphragmatic hernia. *Am J Physiol Lung Cell Mol Physiol.* (2015) 308:L378–83. doi: 10.1152/ajplung.00261.2014

91. Kipfmueller F, Heindel K, Geipel A, Berg C, Bartmann P, Reutter H, et al. Expression of soluble receptor for advanced glycation end products is associated with disease severity in congenital diaphragmatic hernia. *Am J Physiol Lung Cell Mol Physiol.* (2019) 316:L1061–9. doi: 10.1152/ajplung.00359.2018

92. Herrera-Rivero M, Zhang R, Heilmann-Heimbach S, Mueller A, Bagci S, Dresbach T, et al. Circulating microRNAs are associated with pulmonary hypertension and development of chronic lung disease in congenital diaphragmatic hernia. *Sci Rep.* (2018) 8:10735. doi: 10.1038/s41598-018-29153-8

93. Eastwood MP, Deprest J, Russo FM, Wang H, Mulhall D, Iwasiow B, et al. MicroRNA 200b is upregulated in the lungs of fetal rabbits with surgically induced diaphragmatic hernia. *Prenat Diagn.* (2018) 38:645–53. doi: 10.1002/pd.5318

94. Khoshgoo N, Kholdebarin R, Pereira-Terra P, Mahood TH, Falk L, Day CA, et al. Prenatal microRNA miR-200b therapy improves nitrofen-induced pulmonary hypoplasia associated with congenital diaphragmatic hernia. *Ann Surg.* (2019) 269:979–87. doi: 10.1097/SLA.0000000000002595

95. van der Veeken L, Vergote S, Kunpalin Y, Kristensen K, Deprest J, Bruschettini M. Neurodevelopmental outcomes in children with isolated congenital diaphragmatic hernia: a systematic review and meta-analysis. *Prenat Diagn.* (2021) 42:318–29. doi: 10.1002/pd.5916

96. Hassan M, Patel D, LaRusso K, Koclas L, Smith-Morin OTM, Shapiro AJ, et al. Risk stratification helps identify congenital diaphragmatic hernia (CDH) infants in need of formal neurodevelopmental assessment: observations from a structured, interdisciplinary long-term follow-up clinic. *J Pediatr Surg.* (2022) 57:846–50. doi: 10.1016/j.jpedsurg.2021.12.048

97. Lloyd DFA, Rutherford MA, Simpson JM, Razavi R. The neurodevelopmental implications of hypoplastic left heart syndrome in the fetus. *Cardiol Young.* (2017) 27:217–23. doi: 10.1017/S1047951116001645

98. Foglia EE, Ades A, Hedrick HL, Rintoul N, Munson DA, Moldenhauer J, et al. Initiating resuscitation before umbilical cord clamping in infants with congenital diaphragmatic hernia: a pilot feasibility trial. *Arch Dis Child Fetal Neonatal Ed.* (2020) 105:322–6. doi: 10.1136/archdischild-2019-317477

99. Lefebvre C, Rakza T, Weslinck N, Vaast P, Houfflin-debarge V, Mur S, et al. Feasibility and safety of intact cord resuscitation in newborn infants with congenital diaphragmatic hernia (CDH). *Resuscitation.* (2017) 120:20–5. doi: 10.1016/j.resuscitation.2017.08.233

100. Kashyap AJ, Hodges RJ, Thio M, Rodgers KA, Amberg BJ, McGillick EV, et al. Physiologically based cord clamping improves cardiopulmonary haemodynamics in lambs with a diaphragmatic hernia. *Arch Dis Child Fetal Neonatal Ed.* (2020) 105:18–25. doi: 10.1136/archdischild-2019-316906

101. Le Duc K, Mur S, Rakza T, Boukhris MR, Rousset C, Vaast P, et al. Efficacy of intact cord resuscitation compared to immediate cord clamping on cardiorespiratory adaptation at birth in infants with isolated congenital diaphragmatic hernia (Chic). *Children.* (2021) 8:339. doi: 10.3390/children8050339

102. Dekoninck PLJ. Physiological-based cord clamping versus immediate cord clamping for infants born with congenital diaphragmatic hernia: a multicentre, randomised controlled trial. *BMJ Open.* (2022) 12:e054808. doi: 10.1136/bmjopen-2021-054808

103. de Bie FR, Avitabile CM, Joyeux L, Hedrick HL, Russo FM, Basurto D, et al. Neonatal and fetal therapy of congenital diaphragmatic hernia-related pulmonary hypertension. *Arch Dis Child Fetal Neonatal Ed.* (2021) fetalneonatal-2021-322617. doi: 10.1136/archdischild-2021-322617 [Epub ahead of print].

104. Campbell BT, Herbst KW, Briden KE, Neff S, Ruscher KA, Hagadorn JI. Inhaled nitric oxide use in neonates with congenital diaphragmatic hernia. *Pediatrics.* (2014) 134:e420–6.

105. Barrington KJ, Finer N, Pennaforte T, Altit G. Nitric oxide for respiratory failure in infants born at or near term. *Cochrane Database Syst Rev.* (2017) 1:CD000399.

106. Putnam LR, Tsao K, Morini F, Lally PA, Miller CC, Lally KP, et al. Evaluation of variability in inhaled nitric oxide use and pulmonary hypertension in patients with congenital diaphragmatic hernia. *JAMA Pediatr.* (2016) 170:1188–94.

107. Lawrence KM, Monos S, Adams S, Herkert L, Peranteau WH, Munson DA, et al. Inhaled nitric oxide is associated with improved oxygenation in a subpopulation of infants with congenital diaphragmatic hernia and pulmonary hypertension. *J Pediatr.* (2020) 219:167–72. doi: 10.1016/j.jpeds.2019.09.052

108. Kipfmueller F, Schroeder L, Berg C, Heindel K, Bartmann P, Mueller A. Continuous intravenous sildenafil as an early treatment in neonates with congenital diaphragmatic hernia. *Pediatr Pulmonol.* (2018) 53:452–60. doi: 10.1002/ppul.23935

109. Loh E, Stamler JS, Hare JM, Loscalzo J, Colucci WS. Cardiovascular effects of inhaled nitric oxide in patients with left ventricular dysfunction. *Circulation.* (1994) 90:2780–5.

110. Stocker CF, Shekerdemian LS, Nørgaard MA, Brizard CP, Mynard JP, Horton SB, et al. Mechanisms of a reduced cardiac output and the effects of milrinone and levosimendan in a model of infant cardiopulmonary bypass. *Crit Care Med.* (2007) 35:252–9. doi: 10.1097/01.CCM.0000251123.70632.4E

111. Patel N. Use of milrinone to treat cardiac dysfunction in infants with pulmonary hypertension secondary to congenital diaphragmatic hernia: a review of six patients. *Neonatology.* (2012) 102:130–6.

112. Mears M, Yang M, Yoder BA. Is milrinone effective for infants with mild-to-moderate congenital diaphragmatic hernia? *Am J Perinatol.* (2020) 37:258–63. doi: 10.1055/s-0039-1678558

113. Lakshminrusimha S, Keszler M, Kirpalani H, van Meurs K, Chess P, Ambalavanan N, et al. Milrinone in congenital diaphragmatic hernia – a randomized pilot trial: study protocol, review of literature and survey of current practices. *Matern Health Neonatol Perinatol.* (2017) 3:27. doi: 10.1186/s40748-017-0066-9

114. Angadi U, Westrope C, Chowdhry MF. Is levosimendan effective in paediatric heart failure and post-cardiac surgeries? *Interact Cardiovasc Thorac Surg.* (2013) 17:710–4.

115. Schroeder L, Gries K, Ebach F, Mueller A, Kipfmueller F. Exploratory assessment of levosimendan in infants with congenital diaphragmatic hernia. *Pediatr Crit Care Med.* (2021) 22:e382–90. doi: 10.1097/PCC.0000000000002665

116. Acker SN, Kinsella JP, Abman SH, Gien J. Vasopressin improves hemodynamic status in infants with congenital diaphragmatic hernia. *J Pediatr.* (2014) 165:53–8. doi: 10.1016/j.jpeds.2014.03.059

117. Cheung MMH, Smallhorn JF, Redington AN, Vogel M. The effects of changes in loading conditions and modulation of inotropic state on the myocardial performance index: comparison with conductance catheter measurements. *Eur Heart J.* (2004) 25:2238–42. doi: 10.1016/j.ehj.2004.07.034

118. Apitz C, Honjo O, Friedberg MK, Assad RS, van Arsdell G, Humpl T, et al. Beneficial effects of vasopressors on right ventricular function in experimental acute right ventricular failure in a rabbit model. *Thorac Cardiovasc Surg.* (2012) 60:17–23. doi: 10.1055/s-0031-1298058

119. Apitz C, Honjo O, Humpl T, Li J, Assad RS, Cho MY, et al. Biventricular structural and functional responses to aortic constriction in a rabbit model of chronic right ventricular pressure overload. *J Thorac Cardiovasc Surg.* (2012) 144:1494–501. doi: 10.1016/j.jtcvs.2012.06.027

120. Inamura N, Kubota A, Ishii R, Ishii Y, Kawazu Y, Hamamichi Y, et al. Efficacy of the circulatory management of an antenatally diagnosed congenital diaphragmatic hernia: outcomes of the proposed strategy. *Pediatr Surg Int.* (2014) 30:889–94. doi: 10.1007/s00383-014-3574-y

121. Le Duc K, Mur S, Sharma D, Aubry E, Recher M, Rakza T, et al. Prostaglandin E1 in infants with congenital diaphragmatic hernia (CDH) and life-threatening pulmonary hypertension. *J Pediatr Surg.* (2020) 55:1872–8. doi: 10.1016/j.jpedsurg.2020.01.008

122. Lawrence K, Berger K, Herker L, Franciscovich C, O'Dea C, Waqar L, et al. Use of prostaglandin E1 to treat pulmonary hypertension in congenital diaphragmatic hernia. *J Pediatr Surg.* (2019) 54:55–9.

123. Noori S, Friedlich P, Wong P, Ebrahimi M, Siassi B, Seri I. Hemodynamic changes after low-dosage hydrocortisone administration in vasopressor-treated preterm and term neonates. *Pediatrics.* (2006) 118:1456–66. doi: 10.1542/peds.2006-0661

124. Seabrook RB, Grover TR, Rintoul N, Weems M, Keene S, Brozanski B, et al. Treatment of pulmonary hypertension during initial hospitalization in a multicenter cohort of infants with congenital diaphragmatic hernia (CDH). *J Perinatol.* (2021) 41:803–13. doi: 10.1038/s41372-021-00923-z

125. Kamath BD, Fashaw L, Kinsella JP. Adrenal insufficiency in newborns with congenital diaphragmatic hernia. *J Pediatr.* (2010) 156:495–7.e1.

126. Rafat N, Schaible T. Extracorporeal membrane oxygenation in congenital diaphragmatic hernia. *Front Pediatr.* (2019) 7:336. doi: 10.3389/fped.2019.00336

127. Kays DW. ECMO in CDH: is there a role? *Semin Pediatr Surg.* (2017) 26:166–70. doi: 10.1053/j.sempedsurg.2017.04.006

128. Jancelewicz T, Brindle ME, Harting MT, Tolley EA, Langham MR, Lally PA, et al. Extracorporeal membrane oxygenation (ECMO) risk strati fi cation in newborns with congenital diaphragmatic hernia (CDH). *J Pediatr Surg.* (2018) 53:1890–5. doi: 10.1016/j.jpedsurg.2018.04.014

129. Canadian T, Diaphragmatic C, Collaborative H. Diagnosis and management of congenital diaphragmatic hernia: a clinical practice guideline. *Can Med Assoc J.* (2018) 190:E103–12.

130. Tanaka T, Inamura N, Ishii R, Kayatani F, Yoneda A, Tazuke Y, et al. The evaluation of diastolic function using the diastolic wall strain (DWS) before and after radical surgery for congenital diaphragmatic hernia. *Pediatr Surg Int.* (2015) 31:905–10. doi: 10.1007/s00383-015-3766-0

131. Mills J, Safavi A, Skarsgard ED. Chylothorax after congenital diaphragmatic hernia repair: a population-based study. *J Pediatr Surg.* (2012) 47:842–6. doi: 10.1016/j.jpedsurg.2012.01.031

132. Levy SM, Lally PA, Lally KP, Tsao K. The impact of chylothorax on neonates with repaired congenital diaphragmatic hernia. *J Pediatr Surg.* (2013) 48:724–9. doi: 10.1016/j.jpedsurg.2012.11.035

133. Kraemer US, Leeuwen L, Krasemann TB, Wijnen RMH, Tibboel D, IJsselstijn H. Characteristics of infants with congenital diaphragmatic hernia who need follow-up of pulmonary hypertension. *Pediatr Crit Care Med.* (2018) 19:e219–26.

134. Lewis L, Sinha I, Kang SL, Lim J, Losty PD. Long term outcomes in CDH: cardiopulmonary outcomes and health related quality of life. *J Pediatr Surg.* (2022) S0022-3468(22)00251-2. doi: 10.1016/j.jpedsurg.2022.03.020 [Epub ahead of print].

135. Tonelli AR, Plana JC, Heresi GA, Dweik RA. Prevalence and prognostic value of left ventricular diastolic dysfunction in idiopathic and heritable pulmonary arterial hypertension. *Chest.* (2012) 141:1457–65.

136. van Wolferen SA, Marcus JT, Boonstra A, Marques KMJ, Bronzwaer JGF, Spreeuwenberg MD, et al. Prognostic value of right ventricular mass, volume, and function in idiopathic pulmonary arterial hypertension. *Eur Heart J.* (2007) 28:1250–7.

137. Badagliacca R, Poscia R, Pezzuto B, Papa S, Gambardella C, Francone M, et al. Right ventricular dyssynchrony in idiopathic pulmonary arterial hypertension: determinants and impact on pump function. *J Heart Lung Transplant.* (2015) 34:381–9. doi: 10.1016/j.healun.2014.06.010

138. Egan MJ, Husain N, Stines JR, Moiduddin N, Stein MA, Nelin LD, et al. Mid-term differences in right ventricular function in patients with congenital diaphragmatic hernia compared with controls. *World J Pediatr.* (2012) 8:350–4. doi: 10.1007/s12519-012-0380-2

139. Toussaint-Duyster LCC, van der Cammen-van Zijp MHM, de Jongste JC, Tibboel D, Wijnen RMH, Gischler SJ, et al. Congenital diaphragmatic hernia and exercise capacity, a longitudinal evaluation. *Pediatr Pulmonol.* (2019) 54:628–36.

140. Goss KN, Haraldsdottir K, Beshish AG, Barton GP, Watson AM, Palta M, et al. Association between preterm birth and arrested cardiac growth in adolescents and young adults. *JAMA Cardiol.* (2020) 5:910–8. doi: 10.1001/jamacardio.2020.1511

141. Lewandowski AJ, Raman B, Bertagnolli M, Mohamed A, Williamson W, Pelado JL, et al. Association of preterm birth with myocardial fibrosis and diastolic dysfunction in young adulthood. *J Am Coll Cardiol.* (2021) 78:683–92. doi: 10.1016/j.jacc.2021.05.053

142. Lewandowski AJ, Augustine D, Lamata P, Davis EF, Lazdam M, Francis J, et al. Preterm heart in adult life: cardiovascular magnetic resonance reveals distinct differences in left ventricular mass, geometry, and function. *Circulation.* (2013) 127:197–206. doi: 10.1161/CIRCULATIONAHA.112.126920

143. Kinsella JP, Ivy DD, Abman SH. Pulmonary vasodilator therapy in congenital diaphragmatic hernia: acute, late, and chronic pulmonary hypertension. *Semin Perinatol.* (2005) 29:123–8. doi: 10.1053/j.semperi.2005.04.008

144. Zussman ME, Bagby M, Benson DW, Gupta R, Hirsch R. Pulmonary vascular resistance in repaired congenital diaphragmatic hernia vs. age-matched controls. *Pediatr. Res.* (2012) 71:697–700. doi: 10.1038/pr.2012.16

145. Goss KN, Beshish AG, Barton GP, Haraldsdottir K, Levin TS, Tetri LH, et al. Early pulmonary vascular disease in young adults born preterm. *Am J Respir Crit Care Med.* (2018) 198:1549–58.

146. Bojanić K, Grizelj R, Dilber D, Šarić D, Vuković J, Pianosi PT, et al. Cardiopulmonary exercise performance is reduced in congenital diaphragmatic hernia survivors. *Pediatr Pulmonol.* (2016) 51:1320–9.

147. Peetsold MG, Heij HA, Nagelkerke AF, Ijsselstijn H, Tibboel D, Quanjer PH, et al. Pulmonary function and exercise capacity in survivors of congenital diaphragmatic hernia. *Eur Respir J.* (2009) 34:1140–7.

148. Aryal SR, Sharifov OF, Lloyd SG. Emerging role of cardiovascular magnetic resonance imaging in the management of pulmonary hypertension. *Eur Respir Rev.* (2020) 29:190138.

149. Roy CW, van Amerom JFP, Marini D, Seed M, Macgowan CK. Fetal cardiac MRI: a review of technical advancements. *Top Magn Reson Imaging.* (2019) 28:235–44.

150. Zhao D, Lei W, Hu S. Cardiac organoid-a promising perspective of preclinical model. *Stem Cell Res Ther.* (2021) 12:272.

151. DeKoninck P, D'hooge J, van Mieghem T, Richter J, Deprest J. Speckle tracking echocardiography in fetuses diagnosed with congenital diaphragmatic hernia. *Prenat Diagn.* (2014) 34:1262–7. doi: 10.1002/pd.4461

152. Baptista MJ, Nogueira-Silva C, Areias JC, Correia-Pinto J. Perinatal profile of ventricular overload markers in congenital diaphragmatic hernia. *J Pediatr Surg.* (2008) 43:627–33. doi: 10.1016/j.jpedsurg.2007.08.044

153. Zambaiti E, Bussani R, Calcaterra V, Zandonà L, Silvestri F, Peiró JL, et al. Myocardial effects of fetal endoscopic tracheal occlusion in lambs with CDH. *Prenat Diagn.* (2016) 36:362–7. doi: 10.1002/pd.4789

154. Aggarwal S, Shanti C, Lelli J, Natarajan G. Prognostic utility of noninvasive estimates of pulmonary vascular compliance in neonates with congenital diaphragmatic hernia. *J Pediatr Surg.* (2019) 54:439–44. doi: 10.1016/j.jpedsurg.2018.08.057

155. Keller RL, Tacy TA, Hendricks-Munoz K, Xu J, Moon-Grady AJ, Neuhaus J, et al. Congenital diaphragmatic hernia: endothelin-1, pulmonary hypertension, and disease severity. *Am J Respir Crit Care Med.* (2010) 182:555–61. doi: 10.1164/rccm.200907-1126OC

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EDITED BY
Dick Tibboel,
Erasmus Medical Center, Netherlands

REVIEWED BY
Priscilla Pui Lam Chiu,
University of Toronto, Canada
Laurent Storme,
Université de Lille, France

*CORRESPONDENCE
Annabella Braguglia
annabella.braguglia@opbg.net

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Unsolved problems in CDH follow-up

Laura Valfré[1], Andrea Conforti[1], Francesco Morini[2], Neil Patel[3], Francesca Bevilacqua[4], Maria Chiara Cianci[2], Pietro Bagolan[1,5] and Annabella Braguglia[6]*

[1]Neonatal Surgery Unit, Medical and Surgical Department of Fetus-Newborn-Infant, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy, [2]Neonatal Surgery Unit, Meyer Children Hospital, Florence, Italy, [3]Department of Neonatology, Royal Hospital for Children, Glasgow, United Kingdom, [4]Unit of Clinical Psychology, Department of Neuroscience, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy, [5]Department of Systems Medicine, University of Tor Vergata, Rome, Italy, [6]Neonatal Intermediate Care Unit and Follow-Up, Medical and Surgical Department of Fetus-Newborn-Infant, Bambino Gesù Children's Hospital, IRCCS, Rome, Italy

In patients affected by CDH, survival beyond the neonatal period continues to increase thanks to technological and pharmacological improvements. Conversely, patients, families and caregivers are more and more frequently facing "new" complex late comorbidities, including chronic pulmonary and cardiac dysfunctions, neurodevelopmental challenges, and specific nutritional requirements, that often require ongoing long-term medical or surgical care. Therefore, late morbidity is now a key focus in clinical care of CDH. The aims of this paper are to stress some of the most important "unsolved problems" for CDH patients at long-term follow-up.

KEYWORDS

congenital diaphragmatic hernia (CDH), pulmonary hypertension, neurodevelopment, hearing loss, thoracoscopy, problems, long-term follow-up

Introduction

For patients affected by severe CDH survival beyond the neonatal period is continuously improving due to technological and pharmacological improvements in care. A consequence of this are the complex constellation of unique comorbidities, including chronic pulmonary dysfunction, abnormal reactivity of the pulmonary vascular bed, neurodevelopmental challenges, hearing impairment, and nutritional challenges, became more and more frequent, and contributing to long-term medical and surgical care needs.

These longterm challenges may have an important impact on the quality of life (QoL) in CDH, which must be understood by clinicians who treat these children and their families (1).

Therefore, the worldwide focus of interest in CDH care is shifting to late morbidity; including the requirements for standardization of multicenter long-term follow-up programs, comparison of outcomes between centers, and evaluation of the long-term effects of interventions (2).

The aim of this paper is to stress some of the most compelling "unsolved problems" on CDH patients at long-term follow-up (LTFU).

Prenatal assessments, fetal interventions, and long-term outcome

Prenatal unsolved problems focus:

- Prenatal predictors of long-term sequelae.
- Late outcomes of patients underwent prenatal intervention.
- Role of prenatal intervention on right sided CDH.

Prenatal diagnosis and advancements in prenatal and neonatal care have led to improved survival, but the risk of late morbidity remains high (3, 4).

The development and application of prenatal predictors aimed to estimate postnatal outcomes in CDH patients are well established (5). To date, the most widely used are the lung area to head circumference ratio (LHR), and the observed to expected LHR (O/E LHR) obtained using two-dimensional ultrasound (6, 7). Additional predictors have been proposed and investigated including mediastinal shift angle (5). These indices were initially developed with the goal of prenatally predicting postnatal CDH severity and its related mortality risk. With time, technological advancement, and increased standardization, this initial goal progressively shifted toward identification and selection of those most severely affected fetuses to offer fetal intervention (fetal tracheal occlusion, FETO), with the intention to modify post-natal outcomes. Recently, two multicentre randomized studies reported on the results of FETO in different severity-defined groups (8, 9). Despite the time and the efforts of these significant investigations, there is still uncertainty about real benefits of prenatal interventions, as highlighted by different authors (10).

In addition to these short-term two aims, a new ambitious goal has evolved: the ability to prenatally predict long term outcomes in CDH patients surviving the neonatal period.

However, to date, many controversies exist on the ability to prenatally predict long-term morbidity outcomes. In recent papers, prenatal risk stratification based on O/E LHR does not appear to predict a worse outcome in LTFU (11). Specifically, there is no clear association between a lower O/E LHR and a reduction in receptive expressive emergent language test, 3rd Ed. (REEL-3) or Bayley score, nor ventilation/perfusion (V/Q) mismatch. Neonates born with isolated CDH have similar measures of long-term morbidity, including neurological development and growth in height and weight, regardless of their O/E LHR (11).

Similarly, the impact of prenatal intervention on longterm follow-up remains unclear. Intrauterine tracheal occlusion appears to ameliorate and even reverse impaired lung growth in experimental models and in the human condition (8, 9). The technique appears to work by preventing the egress of liquid from the lung, increasing airway pressure, causing cellular proliferation, and increasing alveolar airspace and maturation of pulmonary vasculature.

One recently recognized complication of infants with CDH treated with FETO is tracheomegaly. Recently, McHugh et al. (12) and Zani et al. (13) reported cases of FETO-treated CDH fetuses presenting with respiratory distress shortly after birth, in whom marked tracheomegaly was identified, highlighting potential mechanical airway damage induced by *in utero* balloon occlusion.

Although FETO has a significant impact on tracheal size in CDH infants, the degree of tracheomegaly does not appear to impact survival or need for respiratory support in these infants. Further, the proportion of children with long-term respiratory infections appears to be similar between CDH survivors prenatally treated with FETO, and those who were not (12, 13).

Moreover, role of FETO in right sided CDH (RCDH) infants is poorly characterized: although a greater morbidity in RCDH infants is generally reported, similar mortality was reported in comparison to left CDH patients. Furthermore, considering fetoscopic procedures, in both left and right-sided CDH patients no significant differences in either mortality or short- or longer-term outcomes were reported (14).

CDH, pulmonary hypertension and follow-up

Pulmonary hypertension (PH) unsolved problems focus:

- Natural history of PH beyond neonatal life
- Risk factors for PH at follow-up
- Plasma biomarkers to improve PH assessment
- Impact of new pre- and post-natal therapies

Pulmonary hypertension (PH) is a key component of disease pathophysiology in CDH. Excessive muscularisation and thickening of the pulmonary arterial vessels result in increased pulmonary vascular resistance and pulmonary artery pressure (PAP), and in turn to clinical instability by promoting hypoxic pulmonary-to-systemic shunting, and right (RV) and left (LV) dysfunction (15, 16).

CDH-related PH (CDH-PH) typically resolves in the first weeks of life, persistence beyond this time is associated with increased mortality, ongoing respiratory support and supplemental oxygen in the neonatal period (17–19).

There is limited understanding of the natural history and mechanisms of PH beyond neonatal discharge however, due to the rarity of CDH and challenges of PH assessment.

Longitudinal echocardiographic cohorts have demonstrated PH at discharge in 2–11% of cases, with a trend of ongoing resolution in the first 12 months (17, 20). However,

cross sectional studies using echocardiography and cardiac catheterization have observed PH and RV dysfunction in the second decade of life in some CDH survivors (21–24).

Furthermore, up to 17% CDH cases are discharged on pulmonary vasodilator therapies (25). In the most severe cases chronic or progressive pulmonary vascular disease may contribute to functional restrictions and death in later life (26, 27). For all these reasons CDH-PH follow-up is therefore indicated to monitor PH resolution or progression, guide therapies, and minimize the potential impact on growth, development, functional status and survival.

No reliable risk factors for post-discharge PH have been identified to guide patient selection for follow-up. Wong et al. observed a correlation of fetal lung volumes and PH at 2–5 years, but no such relationship was observed by Fingeret et al. (28) and Wong et al. (29). Empirically, cases with clinical or echocardiographic evidence of PH at discharge or receiving pulmonary vasodilator or oxygen therapy should be routinely followed up from discharge until PH resolution (20).

PH follow-up should be a component of a standardized, multi-disciplinary service, including access to specialist cardiology/PH expertise, and with careful attention to associated factors including nutrition and gastro-oesphageal reflux (30). Assessment and treatment of PH should be in accordance with international guidelines (31–33). Additional investigation, including cardiac catheterization should be guided by cardiology and PH experts in the team.

Many unknowns remain in post-discharge CDH-PH. Prospective multi-center, multi-model studies are needed to understand the pathophysiological mechanisms, risk factors, explore the roles of ventricular function, MRI, and plasma biomarkers for improved assessment, and the impact of new pre- and post-natal therapies (34–36).

Respiratory outcomes

Respiratory unsolved problems focus:

- Natural history of pulmonary function during the long term.
- Predictors of late pulmonary function status in CDH survivors.
- Standardized strategies to reduce late respiratory problems (including RSV immunization to physical activities).

A standardized, multidisciplinary approach to CDH patients is essential to optimize respiratory outcomes at early and late follow-up (37–40).

CDH survivors may present with variable degrees of pulmonary hypoplasia, most often manifesting as recurrent respiratory tract infections (RTI) and/or obstructive symptoms (wheezing/asthma) (41–43). In recent series, the prevalence of RTI in CDH survivors ranges from 10% to over 50%: with an increasing trend of RTI during childhood from 10% at 6 months of age to 23% at 24 months of age. However, there is no evidence of a direct correlation between CDH severity and risk of developing RTI (44).

A recent large retrospective cohort study in CDH survivors observed a progressive decline of average pulmonary function in comparison to normative population standards (9): those with more severe CDH (defined as those with larger type C and D diaphragmatic defects) are at higher risk of deteriorating pulmonary function tests and may benefit from early recognition and monitoring for possible complications. Oxygen requirement at initial hospital discharge also correlated with decreased force expiratory volume by an average of 8.0% (45).

CDH survivors reaching adolescence and early adulthood often present with obstructive pulmonary symptoms, confirmed at spirometry testing (46). Some authors have observed that obstructive respiratory patterns can be detected early in life among CDH survivors and may be used to predict late respiratory outcomes (47). Finally, correlations between late pulmonary obstructive symptoms, neonatal pulmonary hypoplasia, and neonatal pulmonary hypertension have been reported. These findings reflect the intimate relationship between alveolar growth and maturation of the pulmonary vascular bed, both reduced in surviving patients with CDH (22, 46, 47).

Nevertheless, there are no definitive means of stratifying the risk of late pulmonary dysfunction in CDH survivors. This has led to a lack of standardized interventional strategies to reduce late respiratory problems in these patients. This includes a lack of quality evidence in relation to rates of RSV bronchiolitis and appropriate use of palivizumab viral prophylaxis in CDH patients (48).

Gastroesophageal reflux

Gastroesophageal reflux (GER) unsolved problems focus:

- Late consequences of GER in CDH survivors.
- Timing and type of investigations to define GER.
- Treatment options for GER (pharmacological and surgical).

Approximately 60% of congenital diaphragmatic hernia (CDH) survivors present with long term sequelae, including pulmonary, neurological, and gastrointestinal morbidity. One of the most frequently reported disorders is gastroesophageal reflux (GER), which can lead to complications such as esophagitis and Barret esophagus, worsen or contribute to pulmonary morbidity, and is related to failure to thrive (30, 49).

A meta-analysis on patients with CDH performed by Machancoses and collaborators reported an overall prevalence

of gastroesophageal reflux disease (GERD) of 53% in neonates and 35% in infants older than 1 year; a surgical anti-reflux procedure was required in 8–21% of cases. This meta-analysis highlighted a variability in the reported incidence, maybe due to the diagnostic method used. Current follow-up protocols suggest investigating GER only in presence of symptoms, but it may be underdiagnosed in asymptomatic patients if systematic esophageal monitoring is not performed (50–52). Therefore, in relation to the possible consequences of GERD in CDH survivors, Morandi et al. warranted a close follow-up even for asymptomatic patients, but the right timing and type of investigations (endoscopy, pH-impedance monitoring) for asymptomatic patients still needs to be defined (52).

In CDH patients, different mechanisms may contribute to the pathogenesis of GER: esophagogastric junction (EGJ) alteration, weakness of the crura, shortening of the esophagus, abnormal enteric innervation, impaired peristalsis, intestinal malrotation and increased post-surgery abdominal pressure (50, 51, 53). Rayyan et al. hypothesized that EGJ alteration may result from the diaphragmatic defect itself and its surgical treatment (54). Investigating esophageal motility and EGJ function with high-resolution manometry and impedance in CDH patients with and without patch repair, they found that peristaltic motor patterns in patients with CDH were comparable to controls demonstrating that the neural innervation of the esophageal body is preserved. On the other hand, EGJ end-exhalation pressure and inhalation-exhalation pressure difference were lower in patients with CDH primary repaired, suggesting that the activity of the crural diaphragm is reduced and that patch repair tightens the EGJ increasing flow resistance (54).

An optimal management of GERD requires reliable predictors that allow early preventative measures. Different variables were investigated as predictive of GER, both prenatal and postnatal.

Verla observed that larger defects and intrathoracic stomach displacement on prenatal MRI were significantly associated with the diagnosis of GERD, but an intrathoracic liver was not. On the other hand, these variables were not associated with the need of anti-reflux surgery (55). Cordier et al. found that stomach position on prenatal ultrasound was independently associated with GER. A correlation with the duration of parenteral nutrition and the persistence of oral aversion at 2 year was also mentioned (56). Therefore, in addition to predicting overall CDH severity in terms of postnatal mortality, need for prosthetic patch repair and use of extracorporeal membrane oxygenation (ECMO), stomach grading classification is a promising prenatal imaging factor predicting the postnatal occurrence of GER (56).

Fetal endoscopic tracheal occlusion (FETO) was mentioned as a possible factor increasing the risk of GER occurrence, but retrospective multi-center studies performed by Cordier and Leva revealed that the procedure does not impact on global gastrointestinal morbidity at 2 years of age (56, 57).

Several post-natal factors are associated with an increased risk of GER, including right-sided CDH, management with non-conventional mechanical ventilation such as high frequency oscillatory ventilation, need for nitric oxide (NO) and ECMO, the need for patch closure and liver within the chest (55, 58). On multiple variable analysis, however, Diamond et colleagues demonstrated that only liver in the chest and patch repair were significant predictors of GER. Patch repair seemed to be as well an independent predictive of anti-reflux surgery for patient with left-sided CDH (30, 55, 58). On the other hand, Meier and coworkers found no correlation between the incidence of GER and anatomical variations or between the preoperative herniation of the stomach and GER symptoms (51).

Therefore, despite several promising predictors for GERD, both prenatal and postnatal, no definitive and evidence-based predictor exist so far to drive GERD prophylaxis with certainty.

Treatment of GERD is based on pharmacological management with proton pump inhibitors (PPI) (59) and is recommended in CDH survivors during the first year of life. Oral PPI administration, however, presents some issues in infants: considering challenge in oral intake, granules are often crushed with subsequent variable degree of systemic drug exposure and administering suspending formula by gastric tube may lead to tube blockage. To overcome these limitations, Bestebreurtje suggested rectal administration of omeprazole (1 mg/kg), demonstrating results comparable to oral dosing in terms of increasing intra-esophageal and gastric pH (60). This therefore provides a promising alternative administration route for CDH infants with pathological GERD, but further studies are needed to introduce this method in clinical practice.

Despite medical treatment, symptoms of GERD often do not improve, therefore different additional approaches are required including use of nasogastric tubes (in ∼25% of patients), enteral access procedures (gastrostomy or jejunostomy) or anti-reflux surgery (in 6–25% of cases) (50, 58, 61). Nasogastric tubes often complicate the establishment of eventual oral feeding; thus, their use is recommended for a limited period only. Prieto et al. identified characteristics of neonates with CDH independently associated with enteral access procedures during their initial hospitalization: oxygen requirement at 30 days, chromosomal abnormalities, gastroesophageal reflux, major cardiac anomalies, ECMO requirement, liver herniation and increased defect size. Based on these variables the authors established a clinical scoring system which may be considered in counseling and clinical decision making to better predicting the need for enteral access (53).

For patient with intra-thoracic liver and who received patch repair, anti-reflux procedures seem to be the management of choice for GERD and they are most commonly performed in the year after CDH repair (62). Performing fundoplication at a later stage for recalcitrant symptoms is often difficult due to adhesions, the presence of a synthetic patch and abnormal positioning of the spleen and liver (61), thus some authors have

suggested one-step procedure with CDH repair. Few studies have analyzed the impact of preventive fundoplication at the time of CDH repair, suggesting that the procedure is safe and effective in preventing GER and growth disorders in patients with the intermediate or severe anatomical form of CDH and appears to improve post-operative oral feeding (61–63). Conversely, in patients with milder CDH, this approach would appear to prove more challenge than any benefits justify (61–63). Additionally, Meier et al. reported that infants benefit from fundoplication at the time of CDH repair only within the first year of life, while later the difference in GERD symptoms is not statistically significant compared to patients who did not undergo "preventative" anti-reflux surgery (51). Therefore, while intriguing, the role of preventative anti-reflux surgery in CDH patients remains unresolved.

CDH neurodevelopmental outcome

Neurodevelopmental unsolved problems focus:

- Domain and methods to assess neurodevelopmental outcomes.
- Risk factors for neurodevelopmental impairment.

Neurodevelopmental impairment is recognized to be one of the most important sequalae in children born with CDH. Nevertheless, studies had provided only general understanding about neurodevelopmental morbidity and report variable incidence rates. The majority of studies focused on the first 3 years of life, indicating that CDH survivors are at risk for cognitive and motor dysfunction in between 16 and 80% of cases (64). However, there is no consensus regarding the different domains tested, as well as the different methods to test these domains (e.g., time frame, definitions of severity delays, etc.). This variation in testing has prevented a clear definition of possible neurodevelopmental impairment, and its correlation with different potential risk factors.

Nevertheless, despite this ambiguity, many authors agree that gross motor skills domain is the most impaired and least likely to improve (2, 65). Intrathoracic liver position, preterm delivery, 5-min APGAR, prolonged supplemental oxygen requirement, the use of ECMO, prolonged hospitalization, periventricular leukomalacia, initial neuromuscular hypotonicity as well as presence of associated anomalies are the most frequently reported risk factors for late motor impairment (65, 66).

Similar uncertainty is present in studies in preschool and school age CDH survivors. Neurocognitive impairment has been described in percentages varying from 0 to 40% of children (67, 68), while motor abilities appear to remain the most commonly impaired, in particular fine motor coordination, motor planning, and visual processing. Moreover, CDH survivors seem to be at increased risk for developing emotionally reactive and pervasive developmental problems, and higher risk of autism (68, 69).

When considering LTFU, a significantly higher proportion of CDH survivors will not achieve a school degree in comparison with general population. However, among those able to achieve a school degree, school achievements, educational level, and socioeconomic perspective are similar to age and sex-matched healthy controls (70, 71).

Finally, it must be considered that advances in neonatal intensive care, use of extracorporeal membrane oxygenation, and fetal interventions while increasing the chance to survive neonatal period, may contribute to increased burden of late neurodevelopmental morbidities.

Sensorineural hearing loss

SNHL unsolved problems focus:

- Risk factors for SNHL (early and late onset).
- Length of appropriate follow-up.

In patients with CDH, SNHL has been reported with a variable prevalence, ranging from 0 (72) to 100% (73). Earlier studies tend to present a higher prevalence of SNHL, Amoils et al. (74) report a prevalence of SNHL over 50% and Alenazi et al. (75) found SNHL in 7 out of 38 (18%) CDH survivors. Controversies exist on the impact of the diagnosis of CDH *per se* on the risk of SNHL development. In a study on 111 ECMO graduates, Fligor et al. reported a 26% overall prevalence of SNHL in neonates with severe respiratory distress and described CDH as an independent risk factor (76). Conversely, a more recent study of 136 ECMO survivors observed a prevalence of 9% of SNHL, irrespective of the underlying diagnosis (77). As far as the natural history is concerned, in CDH patients, SNHL tends to present as late-onset and progressive. Most studies with data from neonatal hearing screening, report normal findings (73, 74, 78–82). Therefore, the extreme variability in length of follow-up in available reports, precludes firm conclusions on the actual prevalence.

The most frequently reported factors associated with SNHL are ECMO treatment (74, 76, 83, 84), length of mechanical ventilation and/or stay in the NICU or in hospital (74, 79, 80, 84–86), need for inhaled nitric oxide (85), patch repair (74), and dose and duration of specified drugs: loop diuretics (74, 79, 83–85), aminoglycosides (76, 84, 85), and pancuronium bromide (79, 85). Overall, these factors suggest that the most critically ill CDH patients are at greatest risk. On the other hand, Alenazi et al. found no association between CDH disease severity and risk of developing SNHL (75), suggesting that congenital factors may contribute to its development in CDH patients. It is possible that patients with CDH may be congenitally predisposed to a

higher sensitivity to risk factors for SNHL. Identifying definite factors that place CDH patients at high risk for SNHL will permit their modification and may aid prognostication.

Thoracoscopic vs. laparotomic surgery and long-term outcome

Surgical unsolved problems focus:

- Role of minimally invasive surgery.
- Timing of surgical repair in ECMO patients.
- Late surgical sequelae (minimally invasive surgery and open surgery).

Optimal surgical repair of CDH is still highly debated. Minimally invasive surgeries (thoracoscopic and laparoscopic) and open laparotomic approaches were used mostly based on surgeons' beliefs and experiences.

Although the choice between surgical options is poorly evidence-based, there is wide agreement that surgery should be delayed until physiological stability has been achieved and should be performed in elective circumstances (87, 88). Nonetheless, examining ECMO patients, international debate is still ongoing on the uncertainty surrounding optimal timing of CDH repair in infants on ECMO (89): the CDH Euro Consortium admits possible advantage to surgical repair during ECMO, while the Canadian CDH Collaborative and Congenital Diaphragmatic Hernia Study Group (CDHSG) advise delaying surgery until after ECMO weaning (87, 88).

Generally, minimal invasive surgery (MIS) is used in more stable patients, while more severe infants (e.g., those requiring HFOV or ECMO) are treated by open surgical procedures.

No definitive answer has been achieved on optimal surgical treatment, when considering the wide range of surgery-related morbidities reported after CDH repair. These include postoperative small bowel obstruction, feeding difficulties (requiring gastrostomy or fundoplication), and diaphragmatic hernia recurrence (90).

In general, surgical morbidity is directly linked to the method of repair. The major and most frequently reported downside of MIS in CDH repair is the higher risk of recurrence (91–93), reported three- to four-fold higher with the MIS approach. However, there is ongoing no definitive answer on poorer surgical outcomes for MIS, with some recent studies reporting similar recurrence rate between MIS and open repair (94). Furthermore, some authors reported an inverse correlation between risk of recurrence and surgeon's experience, proposing MIS to be limited to high-volume centers and experienced surgeons (95). Finally, the other single risk factor associated with higher recurrence rate is the defect size: it has been suggested to limit MIS to the smallest defects, classified as A or B by the Congenital Diaphragmatic Hernia Study Group (CDHSG) Staging System (93).

Conversely, a large CDHSG data series reported a five-fold increased risk of postoperative adhesive bowel obstruction in open CDH repair, when compared to MIS repair (95), although MIS patients had significantly less severe CDH.

Other Authors reported that up to 20% of CDH survivors may require operative intervention for a small bowel obstruction, regardless of the type of initial surgery, and those patients at increased risk include those who required patch repair (96).

In conclusion, both MIS and open surgery appear to be associated with benefits and weaknesses with no definitive advantages of one over the other.

In conclusion, all the efforts made to improve early survival in CDH patients have progressively shifted substantial attention to late sequelae. Long-term evidence-based data are still lacking, mostly due to the paucity of prospective multicentre studies.

The main unsolved problems in CDH follow-up can be summarized into four main groups:

1. Identification of risk factors (either prenatal or early perinatal) for late pulmonary function, PH, GERD and SNHL.
2. Correlation between prenatal predictors of late outcomes.
3. Characterization of neurodevelopmental outcomes.
4. Optimization of surgical approaches based on patients' clinical characteristics and needed.

The development of different international study groups may help to fill these knowledge gaps, further refining the quality of care offered, and improving patients' longterm quality of life.

Therefore, a possible programme for the next 3–5 years should be focused on optimization LTFU programs by:

- Creating standardized follow-up schedules at different time points, utilizing defined testing, to limit variation between centers.
- Implementing a LTFU international registry.
- Further promoting international multi-center studies.
- Planning a consensus statement on transitional care for CDH patients to adulthood.

To date, there are different international multi-institutional groups focusing their attention on the different topics of the above-mentioned agenda. Specifically, CDH Study Group and CDH Euroconsortium are promoting collaborative studies, implementing treatment guidelines, and exploring new treatments opportunities to improve CDH survival and late quality of life. More recently the European Commission pushed forward the creation of the European Reference Networks (ERNs). The ERNs are virtual networks involving health care providers throughout Europe with the task is to foster discussion about rare or complex conditions and diseases that require highly specialized care and concentrated knowledge

and resources. CDH, a recognized rare and complex disease, is included into the European Reference Network for rare Inherited and Congenital Anomalies (ERNICA). ERNICA is a network (lunched in March 2017) of expert multi-disciplinary healthcare professionals from specialized healthcare providers across Europe aiming to pool together disease-specific expertise, knowledge, and resources otherwise unachievable in a single country. ERNICA aims to reduce health inequalities across Europe, standardizing practices and making high-quality care, by disseminating information and resources to healthcare providers, patients and their families across Europe, regardless of where their geographical localization. To achieve these aims, ERNICA promotes virtual discussion on complex cases, promotes development of "standards of care" (including clinical guidelines and consensus statements), conduction of multi-center high-quality disease-specific research, while developing standardized outcomes measures and data collection.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

LV and AB conceptualized the study. Each author was in charge for his/her own section, writing, editing each chapter. All authors approved final version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Morsberger JL, Short HL, Baxter KJ, Travers C, Clifton MS, Durham MM et al. Parent reported long-term quality of life outcomes in children after congenital diaphragmatic hernia repair. *J Pediatr Surg.* (2019) 54:645–50. doi: 10.1016/j.jpedsurg.2018.06.009

2. Snoek KG, Capolupo I, Braguglia A, Aite L, Van Rosmalen J, Valfrè L, et al. Neurodevelopmental outcome in high-risk congenital diaphragmatic hernia patients: an appeal for international standardization. *Neonatology.* (2015) 109:14–21. doi: 10.1159/000438978

3. Morini F, Valfrè L, Bagolan P. Long-term morbidity of congenital diaphragmatic hernia: a plea for standardization. *Semin Pediatr Surg.* (2017) 26:301–10. doi: 10.1053/j.sempedsurg.2017.09.002

4. Chiu PP, Ijsselstijn H. Morbidity and long-term follow-up in CDH patients. *Eur J Pediatr Surg.* (2012) 22:384–9. doi: 10.1055/s-0032-1329412

5. Romiti A, Viggiano M, Conforti A, Valfré L, Ravà L, Ciofi Degli Atti M, et al. Ultrasonographic assessment of mediastinal shift angle (MSA) in isolated left congenital diaphragmatic hernia for the prediction of postnatal survival. *J Matern Fetal Neonatal Med.* (2020) 33:1330–5. doi: 10.1080/14767058.2018.1517329

6. Alfaraj MA, Shah PS, Bohn D, Pantazi S, O'Brien K, Chiu PP, Gaiteiro R, Ryan G. Congenital diaphragmatic hernia: lung-to-head ratio and lung volume for prediction of outcome. *Am J Obstet Gynecol.* (2011) 205:43.e1–8. doi: 10.1016/j.ajog.2011.02.050

7. Jani J, Nicolaides KH, Keller RL, Benachi A, Peralta CF, Favre R, et al. Observed to expected lung area to head circumference ratio in the prediction of survival in fetuses with isolated diaphragmatic hernia. *Ultrasound Obstet Gynecol.* (2007) 30:67–71. doi: 10.1002/uog.4052

8. Deprest JA, Nicolaides KH, Benachi A, Gratacos E, Ryan G, Persico N, et al. TOTAL trial for severe hypoplasia investigators. Randomized trial of fetal surgery for severe left diaphragmatic hernia. *N Engl J Med.* (2021) 385:107–18. doi: 10.1056/NEJMoa2027030

9. Deprest JA, Benachi A, Gratacos E, Nicolaides KH, Berg C, Persico N, et al. TOTAL Trial for Moderate Hypoplasia Investigators. Randomized trial of fetal surgery for moderate left diaphragmatic hernia. *N Engl J Med.* (2021) 385:119–29. doi: 10.1056/NEJMoa2026983

10. Stolar CJH, Flake AW, Losty PD. Fetal surgery for severe left diaphragmatic hernia. *N Engl J Med.* (2021) 385:2111–2. doi: 10.1056/NEJMc2115673

11. King SK, Alfaraj M, Gaiteiro R, O'Brien K, Moraes T, Humpl T, et al. Congenital diaphragmatic hernia: observed to expected lung to head ratio as a predictor of long-term morbidity. *J Pediatr Surg.* (2016) 51:699–702. doi: 10.1016/j.jpedsurg.2016.02.004

12. McHugh K, Afaq A, Broderick N, Gabra HO, Roebuck DJ, Elliott MJ. Tracheomegaly: a complication of fetal endoscopic tracheal occlusion in the treatment of congenital diaphragmatic hernia. *Pediatr Radiol.* (2010) 40:674–80. doi: 10.1007/s00247-009-1437-9

13. Zani A, Sellars M, Allen P, Tyraskis A, Nicolaides K, Greenough A, et al. Tracheomegaly in infants with severe congenital diaphragmatic hernia treated with fetal endoluminal tracheal occlusion. *J Pediatr.* (2014) 164:1311–5. doi: 10.1016/j.jpeds.2014.02.023

14. Ali K, Dassios T, Khaliq SA, Williams EE, Tamura K, Davenport M, Greenough A. Outcomes of infants with congenital diaphragmatic hernia by side of defect in the FETO era. *Pediatr Surg Int.* (2019) 35:743–7. doi: 10.1007/s00383-019-04484-3

15. Gupta VS, Harting MT. Congenital diaphragmatic hernia-associated pulmonary hypertension. *Semin Perinatol.* (2020) 44:151167. doi: 10.1053/j.semperi.2019.07.006

16. Patel N, Massolo AC, Kipfmueller F. Congenital diaphragmatic hernia-associated cardiac dysfunction. *Sem Perinatol.* (2020)10:1053. doi: 10.1053/j.semperi.2019.07.007

17. Lusk LA, Wai KC, Moon-Grady AJ, Steurer MA, Keller RL. Persistence of pulmonary hypertension by echocardiography predicts short-term outcomes in congenital diaphragmatic hernia. *J Pediatr.* (2015) 166:251–6. doi: 10.1016/j.jpeds.2014.10.024

18. Dillon PW, Cilley RE, Mauger D, Zachary C, Meier A. The relationship of pulmonary artery pressure and survival in congenital diaphragmatic hernia. *J Pediatr Surg.* (2004) 39:307–12. doi: 10.1016/j.jpedsurg.2003.11.010

19. Ferguson DM, Gupta VS, Lally PA, Luco M, Tsao K, Lally KP, et al. Early, postnatal pulmonary hypertension severity predicts inpatient

outcomes in congenital diaphragmatic hernia. *Neonatology*. (2021) 118:147–54. doi: 10.1159/000512966

20. Kraemer US, Leeuwen L, Krasemann TB, Wijnen RMH, Tibboel D, IJsselstijn H. Characteristics of infants with congenital diaphragmatic hernia who need follow-up of pulmonary hypertension. *Pediatr Crit Care Med*. (2018) 19:e219–26. doi: 10.1097/PCC.0000000000001464

21. Filippone NT, Midrio P, Zucchetta P, Svaluto Moreolo G, Toffolutti T, Baraldi E, et al. Cardiopulmonary anatomy and function in long-term survivors of mild to moderate congenital diaphragmatic hernia. *J Pediatr Surg*. (2004) 39:526–31. doi: 10.1016/j.jpedsurg.2003.12.006

22. Trachsel D, Selvadurai H, Bohn D, Langer JC, Coates AL. Long-term pulmonary morbidity in survivors of congenital diaphragmatic hernia. *Pediatr Pulmonol*. (2005) 39:433–9. doi: 10.1002/ppul.20193

23. Zussman ME, Bagby M, Benson DW, Gupta R, Hirsch R. Pulmonary vascular resistance in repaired congenital diaphragmatic hernia vs. age-matched controls. *Pediatr Res*. (2012) 71:697–700. doi: 10.1038/pr.2012.16

24. Husain JRS, Moiduddin N, Stein MA, Nelin LD, Cua CL. Mid-term differences in right ventricular function in patients with congenital diaphragmatic hernia compared with controls. *World J Pediatr*. (2012) 8:350–4. doi: 10.1007/s12519-012-0380-2

25. Behrsin J, Cheung M, Patel N. Sildenafil weaning after discharge in infants with congenital diaphragmatic hernia. *Pediatr Cardiol*. (2013) 34:1844–7. doi: 10.1007/s00246-013-0725-1

26. Iacusso C, Morini F, Capolupo I, Dotta A, Sgrò S, Parisi F, et al. Lung transplantation for late-onset pulmonary hypertension in a patient with congenital diaphragmatic hernia. *Eur J Pediatr Surg Rep*. (2018) 6:1675377. doi: 10.1055/s-0038-1675377

27. Kinsella JP, Ivy DD, Abman SH. Pulmonary vasodilator therapy in congenital diaphragmatic hernia: acute, late, and chronic pulmonary hypertension. *Semin Perinatol*. (2005) 29:123–8. doi: 10.1053/j.semperi.2005.04.008

28. Fingeret L, Thirumoorthi AS, Hahn E, Leskowitz MJ, Aspelund G, Krishnan US, et al. Lung to head ratio in infants with congenital diaphragmatic hernia does not predict long term pulmonary hypertension. *J Pediatr Surg*. (2013) 48:154–7. doi: 10.1016/j.jpedsurg.2012.10.031

29. Wong M, Reyes J, Lapidus-Krol E, Chiang M, Humpl T, Al-Faraj M, et al. Pulmonary hypertension in congenital diaphragmatic hernia patients: Prognostic markers and long-term outcomes. *J Pediatr Surg*. (2018) 53:918–24. doi: 10.1016/j.jpedsurg.2018.02.015

30. Bagolan P, Morini F. Long-term follow up of infants with congenital diaphragmatic hernia. *Semin Pediatr Surg*. (2007) 16:134–44. doi: 10.1053/j.sempedsurg.2007.01.009

31. Rosenzweig EB, Abman SH, Adatia I, Beghetti M, Bonnet D, Haworth S, et al. Paediatric pulmonary arterial hypertension: updates on definition, classification, diagnostics and management. *Eur Respir J*. (2019) 53:1801916. doi: 10.1183/13993003.01916-2018

32. Abman GH, Archer SL, Dunbar Ivy D, Adatia I, Chung WK, Hanna BD, et al. Pediatric pulmonary hypertension: guidelines from the American Heart Association and American Thoracic Society. *Circulation*. (2015) 132:2037–99. doi: 10.1161/CIR.0000000000000329

33. Galiè N, Humbert M, Vachiery JL, Gibbs S, Lang I, Torbicki A, et al. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension. *Eur Heart J*. (2016) 37:67–119. doi: 10.1093/eurheartj/ehv317

34. Avitabile CM, Wang Y, Zhang X, Griffis H, Saavedra S, Adams S, et al. Right ventricular strain, brain natriuretic peptide, and mortality in congenital diaphragmatic hernia. *Ann Am Thoracic Soc*. (2020) 17:1431–9. doi: 10.1513/AnnalsATS.201910-767OC

35. Aryal SR, Sharifov OF, Lloyd SG. Emerging role of cardiovascular magnetic resonance imaging in the management of pulmonary hypertension. *Eur Respir Rev*. (2020) 29:190138. doi: 10.1183/16000617.0138-2019

36. IJsselstijn H, Breatnach C, Hoskote A, Greenough A, Patel N, Capolupo I, et al. Defining outcomes following congenital diaphragmatic hernia using standardised clinical assessment and management plan (SCAMP) methodology within the CDH EURO consortium. *Pediatr Res*. (2018) 84:184–9. doi: 10.1038/s41390-018-0063-3

37. Kays DW. ECMO in CDH: is there a role? *Semin Pediatr Surg*. (2017) 26:166–70. doi: 10.1053/j.sempedsurg.2017.04.006

38. Tsa LRP, Wilson JM, Hirschl RB, Skarsgard ED, Tibboel D, et al. Aggressive surgical management of congenital diaphragmatic hernia: worth the effort? A multicenter, prospective, cohort study. *Ann Surg*. (2017) 267:1. doi: 10.1097/SLA.0000000000002144

39. Lally PA, Skarsgard ED. Congenital diaphragmatic hernia: the role of multi-institutional collaboration and patient registries in supporting best practice. *Semin Pediatr Surg*. (2017) 26:129–35. doi: 10.1053/j.sempedsurg.2017.04.004

40. Puligandla PS, Grabowski J, Austin M, Hedrick H, Renaud E, Arnold M, et al. Management of congenital diaphragmatic hernia: a systematic review from the APSA outcomes and evidence based practice committee. *J Pediatr Surg*. (2015) 50:1958–70. doi: 10.1016/j.jpedsurg.2015.09.010

41. Nobuhara KK, Wilson JM. Pathophysiology of congenital diaphragmatic hernia. *Semin Pediatr Surg*. (1996) 5:234–42.

42. Kosinski P, Wielgos M. Congenital diaphragmatic hernia: pathogenesis, prenatal diagnosis and management: literature review. *Ginekol Pol*. (2017) 88:24–30. doi: 10.5603/GP.a2017.0005

43. Harting MT. Congenital diaphragmatic hernia-associated pulmonary hypertension. *Semin Pediatr Surg*. (2017) 26:147–53. doi: 10.1053/j.sempedsurg.2017.04.008

44. Valfrè L, Braguglia A, Conforti A, Morini F, Trucchi A, Iacobelli BD, et al. Long term follow-up in high-risk congenital diaphragmatic hernia survivors: patching the diaphragm affects the outcome. *J Pediatr Surg*. (2011) 46:52–6. doi: 10.1016/j.jpedsurg.2010.09.061

45. Dao DT, Hayden LP, Buchmiller TL, Kharasch VS, Kamran A, Smithers CJ, et al. Longitudinal analysis of pulmonary function in survivors of congenital diaphragmatic hernia. *J Pediatr*. (2020) 216:158–64. doi: 10.1016/j.jpeds.2019.09.072

46. Wright T, Filbrun A, Bryner B, Mychaliska G. Predictors of early lung function in patients with congenital diaphragmatic hernia. *J Pediatr Surg*. (2014) 49:882–5. doi: 10.1016/j.jpedsurg.2014.01.016

47. Panitch HB, Weiner DJ, Feng R, Perez MR, Healy F, McDonough JM, et al. Lung function over the first 3 years of life in children with congenital diaphragmatic hernia. *Pediatr Pulmonol*. (2015) 50:896–907. doi: 10.1002/ppul.23082

48. Lewis L, Sinha I, Losty PD. Respiratory syncytial virus bronchiolitis in congenital diaphragmatic hernia: A systematic review of prevalence rates and palivizumab prophylaxis. *Pediatr Pulmonol*. (2022) 57:239–44. doi: 10.1002/ppul.25717

49. Power B, Shibuya S, Lane B, Eaton S, De Coppi P. Long-term feeding issue and its impact on the daily life of congenital diaphragmatic hernia survivors: results of the first patient-led survey. *Pediatr Surg Int*. (2020) 36:63–8. doi: 10.1007/s00383-019-04570-6

50. Arcos-Machancoses JV, Ruiz Hernández C, Martin De Carpi J, Pinillos Pisón S. A systematic review with meta-analysis of the prevalence of gastroesophageal reflux in congenital diaphragmatic hernia pediatric survivors. *Dis Esophagus*. (2018) 31:1–12. doi: 10.1093/dote/dox158

51. Maier S, Zahn K, Wessel LM, Schaible T, Brade J, Reinshagen K. Preventive antireflux surgery in neonates with congenital diaphragmatic hernia: a single-blinded prospective study. *J Pediatr Surg*. (2011) 46:1510–5. doi: 10.1016/j.jpedsurg.2011.03.085

52. Morandi A, Macchini F, Zanini A, Pasqua N, Farris G, Canazza L, et al. Endoscopic surveillance for congenital diaphragmatic hernia: unexpected prevalence of silent esophagitis. *Eur J Pediatr Surg*. (2016) 26:291–5. doi: 10.1055/s-0035-1552568

53. Prieto JM, Harting MT, Calvo RY, Carroll JM, Sykes AG, Ignacio RC, et al. Identifying risk factors for enteral access procedures in neonates with congenital diaphragmatic hernia: A novel risk-assessment score. *J Pediatr Surg*. (2021) 56:1130–4. doi: 10.1016/j.jpedsurg.2021.02.029

54. Rayyan M, Omari T, Debeer A, Decaluwe H, Deprest J, Allegaert K, Nathalie R. Characterization of esophageal motility in infants with congenital diaphragmatic hernia using high-resolution manometry. *J Pediatr Gastroenterol Nutr*. (2019) 69:32–8. doi: 10.1097/MPG.0000000000002325

55. Verla MA, Style CC, Mehollin-Ray AR, Fallon SC, Vogel AM, Fernandes CJ, et al. Prenatal imaging features and postnatal factors associated with gastrointestinal morbidity in congenital diaphragmatic hernia. *Fetal Diagn Ther*. (2020) 47:252–60. doi: 10.1159/000501555

56. Cordier AG, Laup L, Letourneau A, Le Sache N, Fouquet V, Senat MV, et al. Prenatal stomach position predicts gastrointestinal morbidity at 2 years in fetuses with left-sided congenital diaphragmatic hernia. *Ultrasound Obstet Gynecol*. (2021) 57:959–67. doi: 10.1002/uog.22086

57. Macchini F, Morandi A, Mazzoleni S, Ichino M, Cavallaro G, Raffaeli G, et al. Is fetal endoscopic tracheal occlusion (FETO) a predisposing factor for acid gastro-esophageal reflux in infants with congenital diaphragmatic hernia? *Front Pediatr*. (2020) 8:1–8. doi: 10.3389/fped.2020.00467

58. Diamond IR, Mah K, Kim PCW, Bohn D, Gerstle JT, Wales PW. Predicting the need for fundoplication at the time of congenital diaphragmatic hernia repair. *J Pediatr Surg*. (2007) 42:1066–70. doi: 10.1016/j.jpedsurg.2007.01.046

59. El-Mahdy MA, Mansoor FA, Jadcherla SR. Pharmacological management of gastroesophageal reflux disease in infants: current opinions. *Curr Opin Pharmacol.* (2017) 37:112–7. doi: 10.1016/j.coph.2017.10.013

60. Bestebreurtje P, de Koning BAE, Roeleveld N, Knibbe CAJ, Tibboel D, van Groen B, et al. Rectal omeprazole in infants with gastroesophageal reflux disease: a randomized pilot trial. *Eur J Drug Metab Pharmacokinet.* (2020) 45:635–43. doi: 10.1007/s13318-020-00630-8

61. Guner YS, Elliott S, Marr CC, Greenholz SK. Anterior fundoplication at the time of congenital diaphragmatic hernia repair. *Pediatr Surg Int.* (2009) 25:715–8. doi: 10.1007/s00383-009-2411-1

62. Dariel A, Rozé JC, Piloquet H, Podevin G, French CDH Study Group. Impact of prophylactic fundoplication on survival without growth disorder in left congenital diaphragmatic hernia requiring a patch repair. *J Pediatr.* (2010) 157:688–91. doi: 10.1016/j.jpeds.2010.06.009

63. Chamond C, Morineau M, Gouizi G, Bargy F, Beaudoin S. Preventive antireflux surgery in patients with congenital diaphragmatic hernia. *World J Surg.* (2008) 32:2454–8. doi: 10.1007/s00268-008-9738-y

64. Van der Veeken L, Vergote S, Kunpalin Y, Kristensen K, Deprest J, Bruschettini M. Neurodevelopmental outcomes in children with isolated congenital diaphragmatic hernia: a systematic review and meta-analysis. *Prenat Diagn.* (2021) 2:e5916. doi: 10.1002/pd.5916

65. Danzer E, Gerdes M, D'Agostino JA, Hoffman C, Bernbaum J, Bebbington MW, et al. Longitudinal neurodevelopmental and neuromotor outcome in congenital diaphragmatic hernia patients in the first 3 years of life. *J Perinatol.* (2013) 33:893–8. doi: 10.1038/jp.2013.47

66. Church JT, Mon R, Wright T, Coughlin MA, Ladino-Torres M, Tapley C, et al. Neurodevelopmental outcomes in CDH survivors: a single institution's experience. *J Pediatr Surg.* (2018) 53:1087–91. doi: 10.1016/j.jpedsurg.2018.02.064

67. Benjamin JR, Gustafson KE, Smith PB, Ellingsen KM, Tompkins KB, Goldberg RN, et al. Perinatal factors associated with poor neurocognitive outcome in early school age congenital diaphragmatic hernia survivors. *J Pediatr Surg.* (2013) 48:730–7. doi: 10.1016/j.jpedsurg.2012.09.026

68. Gerdes JAD, Partridge EA, Hoffman-Craven CH, Bernbaum J, Rintoul NE, Flake AW, et al. Preschool neurological assessment in congenital diaphragmatic hernia survivors: outcome and perinatal factors associated with neurodevelopmental impairment. *Early Hum Dev.* (2013) 89:393–400. doi: 10.1016/j.earlhumdev.2012.12.009

69. Danzer E, Hoffman C, D'Agostino JA, Gerdes M, Bernbaum J, Antiel RM, et al. Neurodevelopmental outcomes at 5years of age in congenital diaphragmatic hernia. *J Pediatr Surg.* (2017) 52:437–43. doi: 10.1016/j.jpedsurg.2016.08.008

70. Mesas Burgos C, Öst E, Ehrén H, Frenckner B. Educational level and socioeconomic status in patients born with congenital diaphragmatic hernia: a population-based study. *J Pediatr Surg.* (2020) 55:2293–6. doi: 10.1016/j.jpedsurg.2019.12.018

71. Morini F, Lally KP, Lally PA, Crisafulli RM, Capolupo I, Bagolan P. Treatment strategies for congenital diaphragmatic hernia: change sometimes comes bearing gifts. *Front Pediatr.* (2017) 5:195. doi: 10.3389/fped.2017.00195

72. Jaillard SM, Pierrat V, Dubois A, Truffert P, Lequien P, Wurtz AJ, et al. Outcome at 2 years of infants with congenital diaphragmatic hernia: a population-based study. *Ann Thorac Surg.* (2003) 75:250–56. doi: 10.1016/S0003-4975(02)04278-9

73. Robertson CM, Tyebkhan JM, Hagler ME, Cheung PY, Peliowski A, Etches PC. Late-onset, progressive sensorineural hearing loss after severe neonatal respiratory failure. *Otol Neurotol.* (2002) 23:353–6. doi: 10.1097/00129492-200205000-00022

74. Amoils M, Janik MC, Lustig LR. Patterns and predictors of sensorineural hearing loss in children with congenital diaphragmatic hernia. *JAMA Otolaryngol Head Neck Surg.* (2015) 141:923–6. doi: 10.1001/jamaoto.2015.1670

75. Alenazi A, Derraugh G, Levesque M, Morris MI, Shawyer AC, Lum Min SA, et al. The prevalence of hearing loss in children with congenital diaphragmatic hernia: a longitudinal population-based study. *J Pediatr Surg.* (2021) 56:226–9. doi: 10.1016/j.jpedsurg.2020.08.008

76. Fligor BJ, Neault MW, Mullen CH, Feldman HA, Jones DT. Factors associated with sensorineural hearing loss among survivors of extracorporeal membrane oxygenation therapy. *Pediatrics.* (2005) 115:1519–28. doi: 10.1542/peds.2004-0247

77. van den Hondel D, Madderom MJ, Goedegebure A, Gischler SJ, Mazer P, Tibboel D. Sensorineural hearing loss and language development following neonatal extracorporeal membrane oxygenation. *Pediatr Crit Care Med.* (2013) 14:62–9. doi: 10.1097/PCC.0b013e31825b54ae

78. Cortes RA, Keller RL, Townsend T, Harrison MR, Farmer DL, Lee H, et al. Survival of severe congenital diaphragmatic hernia has morbid consequences. *J Pediatr Surg.* (2005) 40:36–46. doi: 10.1016/j.jpedsurg.2004.09.037

79. Masumoto K, Nagata K, Uesugi T, Yamada T, Taguchi T. Risk factors for sensorineural hearing loss in survivors with severe congenital diaphragmatic hernia. *Eur J Pediatr.* (2007) 166:607–12. doi: 10.1007/s00431-006-0300-3

80. Javidnia H, Vaccani JP. Progressive sensorineural hearing loss in children with congenital diaphragmatic hernias. *J Otolaryngol Head Neck Surg.* (2009) 38:29–31.

81. Morando C, Midrio P, Gamba P, Filippone M, Sgrò A, Orzan E. Hearing assessment in high-risk congenital diaphragmatic hernia survivors. *Int J Pediatr Otorhinolaryngol.* (2010) 74:1176–9. doi: 10.1016/j.ijporl.2010.07.009

82. Wilson MG, Riley P, Hurteau AM, Baird R, Puligandla PS. Hearing loss in congenital diaphragmatic hernia (CDH) survivors: is it as prevalent as we think? *J Pediatr Surg.* (2013) 48:942–5. doi: 10.1016/j.jpedsurg.2013.02.007

83. Rasheed A, Tindall S, Cueny DL, Klein MD, Delaney-Black V. Neurodevelopmental outcome after congenital diaphragmatic hernia: extracorporeal membrane oxygenation before and after surgery. *J Pediatr Surg.* (2001) 36:539–44. doi: 10.1053/jpsu.2001.22278

84. Partridge EA, Bridge C, Donaher JG, Herkert LM, Grill E, Danzer E, et al. Incidence and factors associated with sensorineural and conductive hearing loss among survivors of congenital diaphragmatic hernia. *J Pediatr Surg.* (2014) 49:890–4. doi: 10.1016/j.jpedsurg.2014.01.019

85. Morini F, Capolupo I, Masi R, Ronchetti MP, Locatelli M, Corchia C, et al. Hearing impairment in congenital diaphragmatic hernia: the inaudible and noiseless foot of time. *J Pediatr Surg.* (2008) 43:380–4. doi: 10.1016/j.jpedsurg.2007.10.048

86. Dennett KV, Fligor BJ, Tracy S, Wilson JM, Zurakowski D, Chen C. Sensorineural hearing loss in congenital diaphragmatic hernia survivors is associated with postnatal management and not defect size. *J Pediatr Surg.* (2014) 49:895–9. doi: 10.1016/j.jpedsurg.2014.01.049

87. Puligandla PS, Skarsgard ED, Offringa M, Adatia I, Baird R, Bailey JM, et al. Diagnosis and management of congenital diaphragmatic hernia: a clinical practice guideline. *CMAJ.* (2018) 190:E103–12. doi: 10.1503/cmaj.170206

88. Snoek KG, Reiss IK, Greenough A, Capolupo I, Urlesberger B, Wessel L, et al. Standardized postnatal management of infants with congenital diaphragmatic hernia in Europe: the CDH EURO Consortium Consensus. 2015 Update. *Neonatology.* (2016) 110:66–74. doi: 10.1159/000444210

89. Kirby E, Keijer R. Congenital diaphragmatic hernia: current management strategies from antenatal diagnosis to long-term follow-up. *Pediatr Surg Int.* (2020) 36:415–29. doi: 10.1007/s00383-020-04625-z

90. Hollinger LE, Buchmiller TL. Long term follow-up in congenital diaphragmatic hernia. *Sem Perinatol.* (2020) 44:151171. doi: 10.1053/j.semperi.2019.07.010

91. Alam PDL, Jesudason EC. Neonatal endosurgical congenital diaphragmatic hernia repair: a systematic review and meta-analysis. *Ann Surg.* (2010) 252:20–6. doi: 10.1097/SLA.0b013e3181dca0e8

92. Terui K, Nagata K, Ito M, Yamoto M, Shiraishi M, Taguchi T, et al. Surgical approaches for neonatal congenital diaphragmatic hernia: a systematic review and meta-analysis. *Pediatr Surg Int.* (2015) 31:891–7. doi: 10.1007/s00383-015-3765-1

93. Cioci AC, Urrechaga EM, Parreco J, Remer LF, Cowan M, Perez EA, et al. One-year outcomes of congenital diaphragmatic hernia repair: factors associated with recurrence and complications. *J Pediatr Surg.* (2021) 56:1542–6. doi: 10.1016/j.jpedsurg.2020.09.018

94. Okawada M, Ohfuji S, Yamoto M, Urushihara N, Terui K, Nagata K, et al. Thoracoscopic repair of congenital diaphragmatic hernia in neonates: findings of a multicenter study in Japan. *Surg Today.* (2021) 51:1694–702. doi: 10.1007/s00595-021-02278-6

95. Putnam LR, Gupta V, Tsao K, Davis CF, Lally PA, Lally KP, et al. Factors associated with early recurrence after congenital diaphragmatic hernia repair. *J Pediatr Surg.* (2017) 52:928–32. doi: 10.1016/j.jpedsurg.2017.03.011

96. Janssen S, Heiwegen K, van Rooij IA, Scharbatke H, Roukema J, de Blaauw I, et al. Factors related to long-term surgical morbidity in congenital diaphragmatic hernia survivors. *J Pediatr Surg.* (2018) 53:508–12. doi: 10.1016/j.jpedsurg.2017.05.032

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EDITED BY
Dick Tibboel,
Erasmus Medical Center, Netherlands

REVIEWED BY
Florian Kipfmueller,
University Hospital Bonn, Germany
Isabella Fabietti,
Bambino Gesù Children's Hospital (IRCCS), Italy

*CORRESPONDENCE
Beverley Power
✉ bev@cdhuk.org.uk

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The CDH patient perspective journey

Beverley Power*

Management Committee, CDH UK – The Congenital Diaphragmatic Hernia Charity, King's lynn, United Kingdom

Background: Congenital Diaphragmatic Hernia is a malformation of the diaphragm resulting in ongoing clinical symptoms and problems. Mortality remains high, particularly where there are other issues involved. Tracking a patient throughout their lifetime to understand the full impact on health and function is challenging. CDH UK is a registered charity supporting anyone affected by CDH. It has over 25 years of experience and a broad range of patient experience and knowledge.
Aims: To develop a patient journey with timepoints of significance.
Methods: We studied our own data and looked at what we already knew from publications and medical advisors. We recruited a focus group, plotted out stages and timepoints through their "lived" experiences using the Team Idea Mapping method. We then compared these experiences to our own data, to identify the common issues in daily life and care.
Outcome: We have developed a patient journey through the eyes of the patient and turned it into a patient friendly infographic. This can be used as a tool to help understand the CDH Journey throughout a patient's lifetime. CDH UK has already used this to create a first prototype of a mobile application. It has also further helped to recognize areas of patient concern and to improve services and resources.
Discussion: This can be used as a basis for care and research, including standards, benchmarking, transition and helping improvements in healthcare, education, family life and social settings. Potentially holding clues as to the etiology and pathology of the condition and an opportunity to further explore theories and unanswered questions. It may help improve counselling and bereavement care, resulting in better general and mental health outcomes.

KEYWORDS

CDH, Congenital Diaphragmatic Hernia, patient journey, outcomes, pediatrics, healthcare, neonatal, transition of care

Introduction

Congenital Diaphragmatic Hernia is a malformation of the diaphragm resulting in long lasting clinical symptoms and problems, that is still poorly understood in terms of long-term outcome (1, 2).

One of the main difficulties of the care of patients with this condition is tracking their progress throughout their lifetime to understand the pathology, to preserve, prevent, and improve health, with the aim of effecting a good quality of life. Transition of care from the pediatric care setting to an adult care setting can be problematic, with little or no planning in the pediatric setting. This often results in poor health outcomes later in life due in part to a lack of knowledge and experience of the condition, particularly in the General Practice healthcare setting.

CDH UK is a registered UK charity that supports anyone affected by CDH, or who has an interest in Congenital Diaphragmatic Hernia. It was founded in 1994 as an informal support group and registered as a charity with the Charity Commission for England and Wales in 2004 (registration

number 1106065) and in Scotland in 2011 (registration number SC042410). The services and resources of CDH UK are accessed by thousands of individuals meaning that the charity has access to a large cohort of patients and carers, which results in a good overview and understanding of patient experiences, needs and priorities. This is mainly acquired due to voluntary patient reported outcomes.

In 2014 CDH UK began thinking about developing a mobile application for patients and families to facilitate patient reported outcomes and to enable them to input and retrieve day to day data. This culminated in approaching developers specializing in mobile applications for patient use. In 2016 we were asked to provide a patient journey by a chosen developer to plot out the relevant time points for the basis of the mobile application, but we realized that there was no published patient journey for CDH and certainly not from the perspective of the patient.

It became clear that not only did we need to know the journey for our mobile application development, but for other reasons such as improving our support services and resources and for research and study too: particularly with the advancement of data collection and technology. Research will most likely benefit from understanding the whole lifespan of a patient journey. Healthcare professionals often do not have information on their patient for a lifetime, as they either discharge in early life, or lose track of the patient during transition, or for other reasons. Neither do they have "lived" experience of Congenital Diaphragmatic Hernia. Therefore, information on a patient's journey will be beneficial for planning transition of care and understanding any potential future health problems that their patient may experience.

This poses the question "What does the CDH patient perspective Journey look like?"

We aimed to develop a patient perspective Journey, mapping out timepoints of significance in a patient's lifetime referencing points of care, wellbeing and social aspects that depicts what happens; what we know happens, what we think we know happens and what we would like to see happen, and at what timepoints in life.

Methods

The CDH Patient Perspective journey was created using a mixed method of Qualitative and Quantitative research carried out in 4 stages.

Stage 1

We studied our own historical data that was less than 10 years old and that was collected through various means as follows:

1. Online surveys using Survey Monkey™.
2. Posts and comments on our Social Media accounts and groups
3. Face to face conversations at Get Together meetings
4. Online events
5. Support line calls
6. Emails into the support inbox

The data analyzed was derived from various ages of individuals falling in to three categories:

1. Parents (biological or non-biological)
2. Carers (Any person involved in the care of the patient or their family other than parents)
3. Patients (the person suffering with a Congenital Diaphragmatic Hernia or eventration)

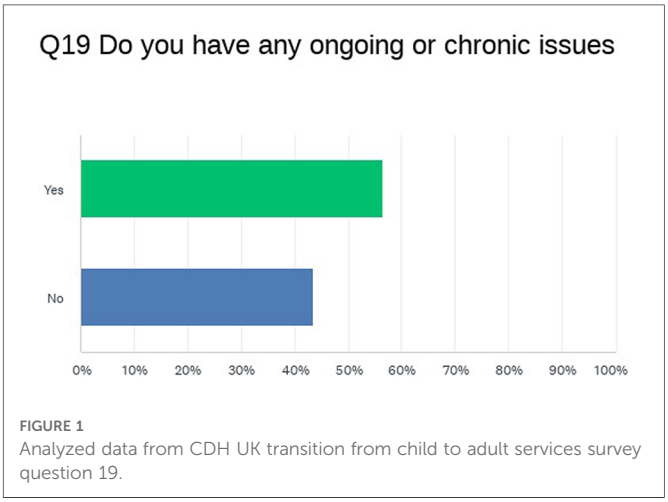

FIGURE 1
Analyzed data from CDH UK transition from child to adult services survey question 19.

The aim was to look for problems reported, or common requests for support, to enable us to understand common themes in care, health, or quality of life issues.

We also considered what we already knew from publications, attending conferences and from discussions with our medical advisors and Patrons.

The example below is of data captured and analyzed from twenty-three adult respondents of one question within a survey regarding transition of care (**Figure 1**).

Stage 2

A focus group of thirteen parents and other family members of mixed sex and ages was recruited by approaching our members. Nine members of the group met face to face (two virtually) for a full afternoon workshop to plot out the various stages and timepoints through their "lived" experiences using the Team Idea Mapping Method (3), which allowed us to create a flow map of different scenarios (**Figure 2**). This included Antenatal diagnosis, postnatal diagnosis before discharge, after birth, bereavement, and post discharge. We also added a list of data capture points at the end of the flow map.

Stage 3

We compared the flow map to experiences of the patients and families that we have supported over the years using data derived from Stage 1. We considered examples of patient reported outcomes of care that were below what the patients or parents expected and examples of good practices and good care according to patients and parents, but not necessarily according to literature, or local healthcare guidelines.

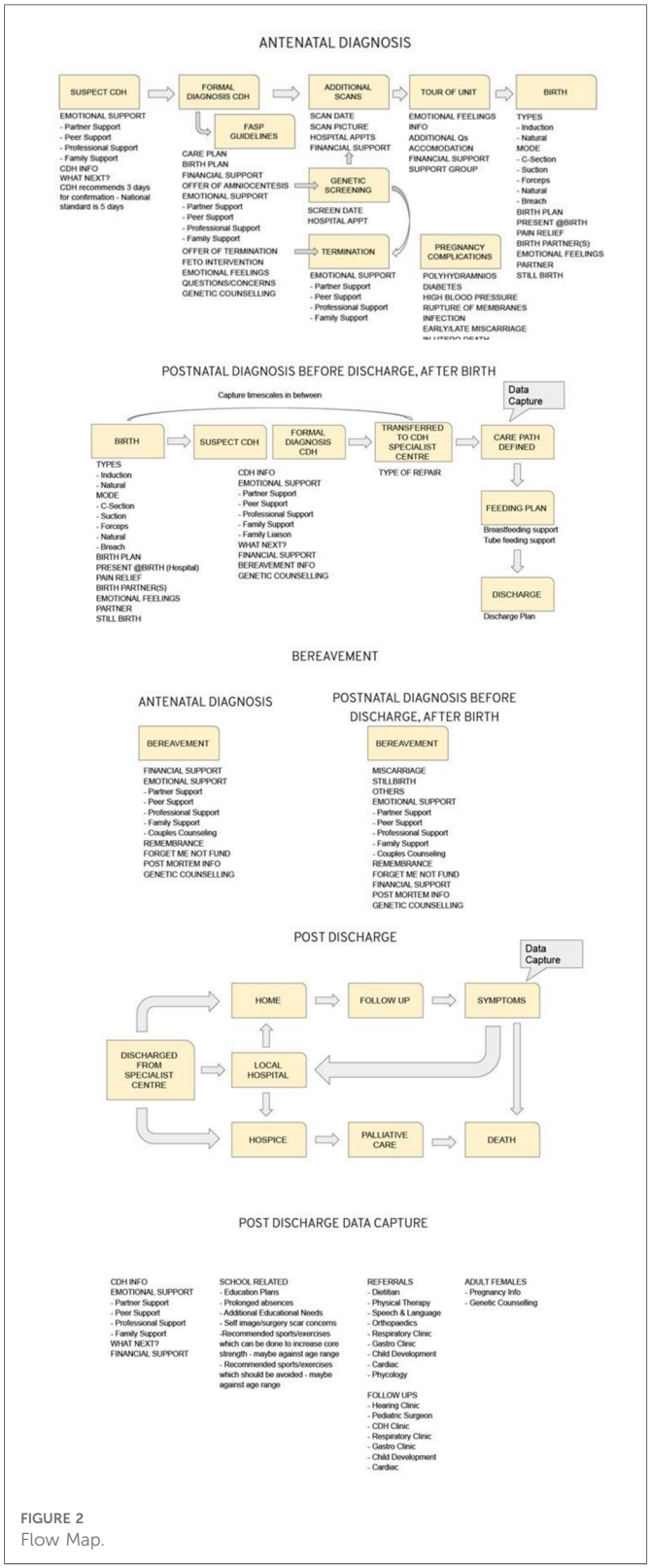

FIGURE 2
Flow Map.

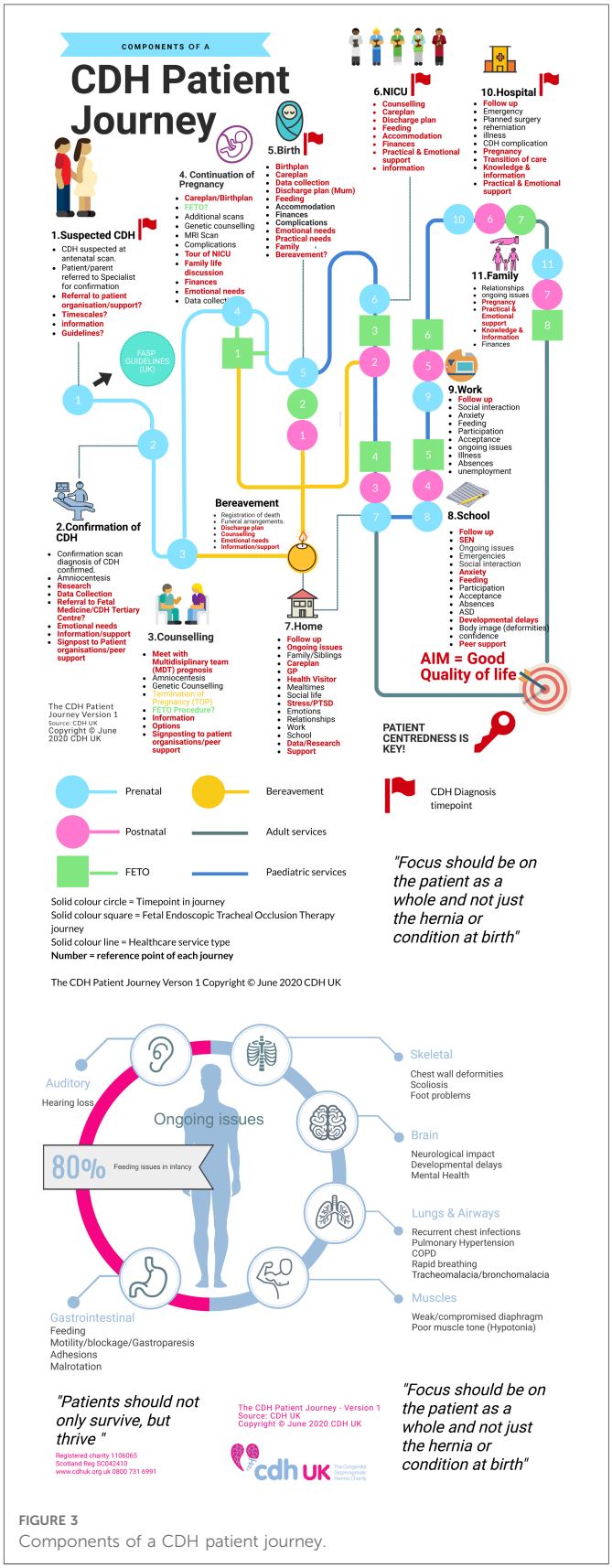

FIGURE 3
Components of a CDH patient journey.

We created a visual using a paid creative license titled "Components of a CDH Patient Journey" (Figure 3). This visual was presented at our Great Get Together online event in June 2020, but feedback proved that this visual is too complex to follow and could not be used as an infographic for lay persons.

We asked a group of over twenty families, patients and caregivers to carry out a further review of the first visual (Figure 3) to help to reduce the text and simplify the visual. The group provided a table of amendments (Figure 4).

Timepoint>	Diagnosis	Pregnancy	Birth	Hospital	Home	Childhood/Adulthood	Bereavement
What Happens when? **Please note**-points not necessarily in number order or limited	1. Imaging 2. Amniocentesis offered 3.CDH confirmed 4.Referral to specialist Centre 5.Counselling 6.Termination offered 7.Data captured	1. Counselling 2.Genetic testing 3.Further scans 4.Complications 5.Fetal Therapy (FETO) if suitable 6.Termination of Pregnancy if opted.	1.Induction or spontaneous labour 2.Baby treated post birth 3. Baby transferred to on-site NICU or another specialist unit 4.Still birth	1.Birth Mother discharged 2.Baby in NICU/PICU 3.Weaning off ventilator/oxygen/medications 4.Feeding established 5.Baby discharged to local hospital or home 6.Data captured	1. Outreach nurses 2. Postnatal care 3. Follow up appointments 4. Symptoms	1.Ongoing issues varied in type and severity 2. Symptoms 3.Reherniation 4.childhood ailments 5.Transition from child to adult services 6.Hospital appointments/admission	1.Unexpected death during pregnancy or after birth 2.Termination of Pregnancy 3.stillbirth 4.Grieving process 5.Post-mortem offered
Challenges	1.Awareness 2. Choices 3.Anxiety & fear 4.Interpretation of information. 5.Outcomes	1.Decision making 2.Birth plan 3.Care plan 4.Mental health 5.Inclusivity 6.Complications 7.Bereavement	1.Options 2.Labour 3.Bonding 4.Mental Health 5.Recovery 6.Bereavement	1.Postnatal care 2.Mental Health 3.Self-care 4.Feeding 5.Bonding 6.Family & friends 7.Environment 8.Work/school 9. Finances 10.Bereavement	1.Ongoing medical needs 2.Awareness/lack of knowledge 3.Isolation 4.Mental Health 5.Finances 6.Family dynamics 7.Loss of confidence 8.Data capture/Research	1.Transition of care 2.General/mental Health 3.Feeding & nutrition 4.Body image 5.Awareness 6.Access to care & knowledge 7.Isolation 8.School/Work 9.Relationships 10.Pregnancy & Birth 11.Exercise & social activities 12.Data capture/Research	1. Lack of services 2.Follow up 3.Mental Health 4.Delayed grief 5.Isolation 6.Relationships 7.Family & Friends 8.Family Planning 9.Post-mortem 10.Data capture/Research

FIGURE 4
Table of amendments.

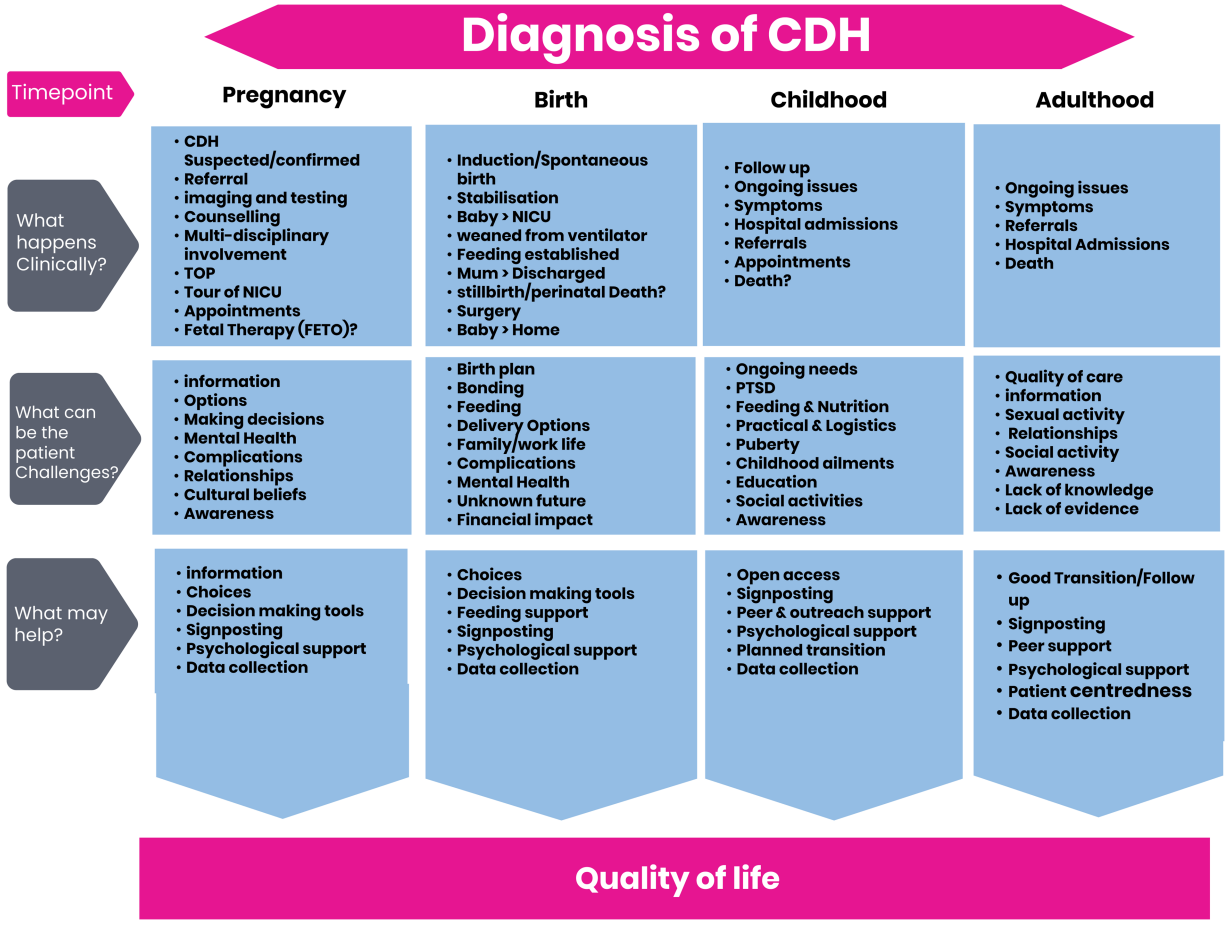

FIGURE 5
CDH Patient Journey chart.

Stage 4

An infographic chart (**Figure 5**) and patient friendly infographic (**Figure 6**) was developed using the feedback from this further group review. The consensus was reached by the described four stages that involved patients, family members, experts in CDH and graphic designers. The patient friendly infographic is depicted as "A rollercoaster journey" as this is often how parents and patients describe it. This is simpler in its form than the earlier version depicted in The Components of a CDH Patient Journey (**Figure 3**).

Ethical considerations

We considered all ethical issues during our research. No ethical approvals were sought as no personally identifiable data is used in our data or reporting. All focus groups were created voluntarily and all participants in our surveys and focus groups were free to withdraw at any time without having to give a reason. There were no payments or recompense given for face-to-face focus group meetings as these took place alongside other meetings and so require no additional out of pocket expense to the group members.

Results

We have identified what the CDH patient Journey looks like for the parents and patient in terms of their lived experience with an antenatal or postnatal diagnosis of CDH, or a diagnosis in later life. Using the information derived from the research stages, we have developed a visual infographic of the patient perspective journey that can be used by patients, caregivers, and researchers (for example), due to the various formats that can be produced from the flow chart. This will provide an insight and help the reader to better understand the CDH Patient Journey throughout a patient's lifetime and to make them aware of the potential health, social, economic, and logistical issues that may impact the patient or family. We recognize that the journey has some limitations; there are a spectrum of case presentations seen with Congenital Diaphragmatic Hernia, with not all cases presented or diagnosed during pregnancy, at birth, or soon after, and so ongoing issues may not be diagnosed early enough to impact on outcomes. Also, with new management strategies and treatments this journey may change, and so periodic review is necessary to reflect up to date patient experiences. In addition, the data is derived from personal experiences which may have been influenced by various other factors.

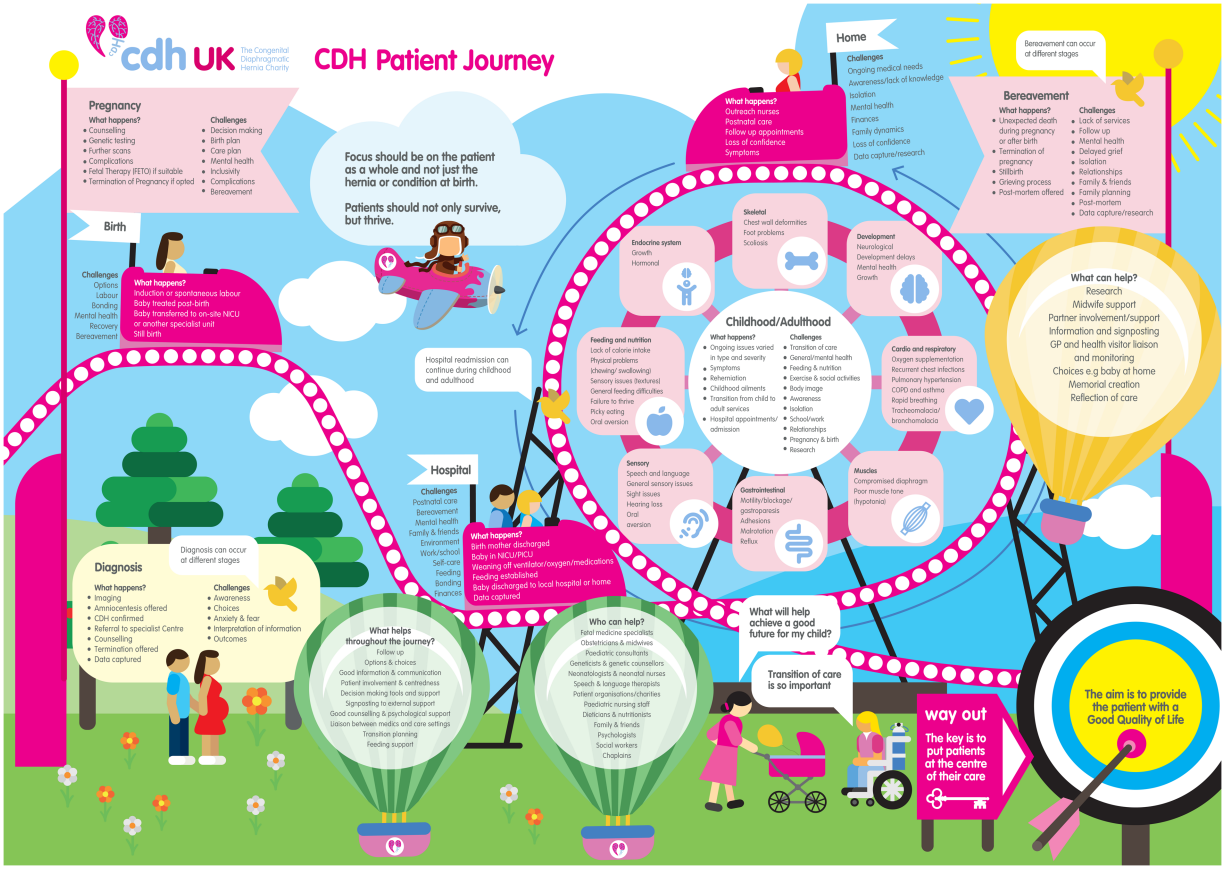

FIGURE 6
CDH Patient Journey V1 infographic.

Discussion

The developed patient journey chart can be used as a basis for care in the UK and beyond, including developing standards, benchmarking, and improvements in care. We also hope that it can be useful for research and will be an instigator for new translational research (4).

The Patient Journey is an ever evolving one, due in part to advances in treatments, care, support, and technology. We therefore realize that the CDH Patient Perspective journey must be reviewed regularly and suggest every two years to ensure it is as accurate and as relevant as possible. We have developed a strategy to ensure that the information that CDH UK produces is of a certain standard and quality and that its lifecycle is maintained.

This CDH Patient Perspective journey may also hold clues as to the etiology and pathology of the condition and could harness an opportunity to further explore theories and unanswered questions. There is also evidence of improved survival rates in severe Congenital Diaphragmatic Hernia (5) and left sided cases (6), which will impact on health services. It may also serve as a basis for the improvement in counselling and bereavement care, resulting in better mental health outcomes for both patients and families.

We have used this patient journey already in its raw form, to create a prototype for a mobile application. We hope in the short term the current Patient Journey Version 1 will serve as a useful support resource for patients, families, and caregivers and in the long term will help with research.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

The First author BP was responsible for the organisation of the project and the study design, data collection and analysis. The writing of the manuscript and submission.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Zimmer J, Puri P. Congenital diaphragmatic hernia. In: P Puri, editors. *Pediatric surgery*. Berlin, Heidelberg: Springer (2017). p. 797–815. doi: 10.1007/978-3-642-38482-0_57-1

2. Tan JK, Banton G, Minutillo C, Hall GL, Wilson A, Murray C, et al. Long-term medical and psychosocial outcomes in congenital diaphragmatic hernia survivors. *Arch Dis Child*. (2019) 104(8):761–7. doi: 10.1136/archdischild-2018-316091

3. Goswami B, Mahajan R, Jain A, Koner BC. Team idea mapping method: A brainstorming session for enhancing problem-solving skills in postgraduate medical biochemistry students as assessed by self-efficacy. *J Med Sci & Res*. (2021) 3(2):74–8. doi: 10.25259/AUJMSR_23_2021

4. Friedmacher F, Pakarinen MP, Rintala RJ. Congenital diaphragmatic hernia: a scientometric analysis of the global research activity and collaborative networks. *Pediatr Surg Int*. (2018) 34(9):907–17. doi: 10.1007/s00383-018-4304-7

5. Gien J, Kinsella JP, Behrendt NJ, Zaretsky MV, Galan HL, Liechty KW. Improved survival for infants with severe congenital diaphragmatic hernia. *J Perinatol*. (2022) 42:1189–94. doi: 10.1038/s41372-022-01397-3

6. Yang MJ, Fenton S, Russell K, Yost CC, Yoder BA. Left-sided congenital diaphragmatic hernia: can we improve survival while decreasing ECMO? *J Perinatol*. (2020) 40(6):935–42. doi: 10.1038/s41372-020-0615-3

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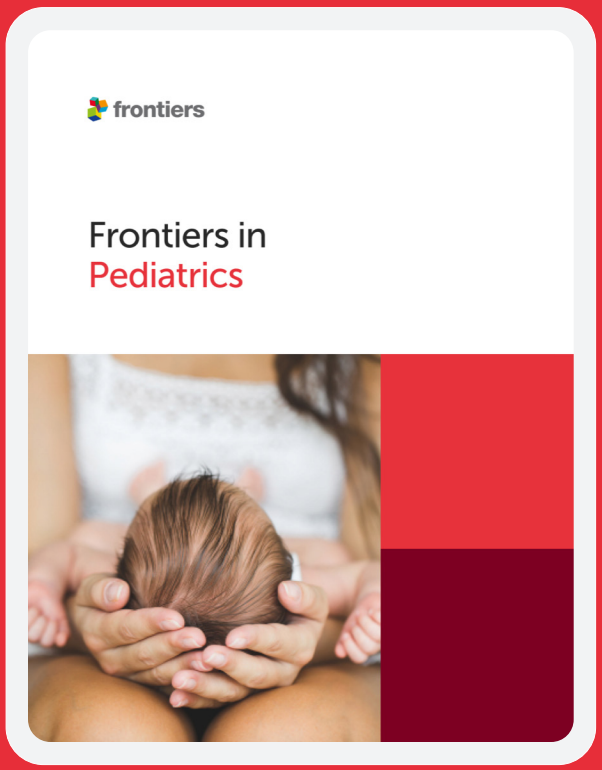

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